



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification 5:</b> C07K 13/00, C12P 21/00, C12N 5/00, 15/00	<b>A1</b>	<b>(11) International Publication Number:</b> WO 94/21679 <b>(43) International Publication Date:</b> 29 September 1994 (29.09.94)
<b>(21) International Application Number:</b> PCT/US94/01957 <b>(22) International Filing Date:</b> 25 February 1994 (25.02.94) <b>(30) Priority Data:</b> 038,769 25 March 1993 (25.03.93) US <b>(60) Parent Application or Grant</b> <b>(63) Related by Continuation</b> US 038,769 (CIP) Filed on 25 March 1993 (25.03.93) <b>(71) Applicant (for all designated States except US):</b> MERCK & CO., INC. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> KENDALL, Richard, L. [US/US]; 5201 Stonehedge Road, Edison, NJ 08820 (US). THOMAS, Kenneth, A., Jr. [US/US]; 245 Washington Avenue, Chatham Borough, NJ 07928 (US). <b>(74) Agent:</b> WALLEN, John, W., III; 126 East Lincoln Avenue, Rahway, NJ 07065 (US).		<b>(81) Designated States:</b> AU, BB, BG, BR, BY, CA, CN, CZ, FI, HU, JP, KR, KZ, LK, LV, MG, MN, MW, NO, NZ, PL, RO, RU, SD, SK, UA, US, UZ, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> INHIBITOR OF VASCULAR ENDOTHELIAL CELL GROWTH FACTOR		
<b>(57) Abstract</b>  The vascular endothelial cell growth factor (VEGF) inhibitors of the present invention are naturally occurring or recombinantly engineered soluble forms with or without a C-terminal transmembrane region of the receptor for VEGF, a very selective growth factor for endothelial cells. The soluble forms of the receptors will bind the growth factor with high affinity but do not result in signal transduction. These soluble forms of the receptor bind VEGF and inhibit its function.		

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

5

- 1 -

10 TITLE OF THE DISCLOSURE

INHIBITOR OF VASCULAR ENDOTHELIAL CELL GROWTH FACTOR

BACKGROUND OF THE DISCLOSURE

Recently a new class of cell-derived dimeric  
15 mitogens with selectivity for vascular endothelial  
cells has been identified and designated vascular  
endothelial cell growth factor (VEGF). VEGF has been  
purified from conditioned growth media of rat glioma  
cells [Conn et al., (1990), Proc. Natl. Acad. Sci.  
20 U.S.A., 87, pp 2628-2632]; and conditioned growth media  
of bovine pituitary folliculo stellate cells [Ferrara  
and Henzel, (1989), Biochem. Biophys. Res. Comm., 161,  
pp. 851-858; Gozpadorowicz et al., (1989), Proc. Natl.  
Acad. Sci. U.S.A., 86, pp. 7311-7315] and conditioned  
25 growth medium from human U937 cells [Connolly, D. T. et  
al. (1989), Science, 246, pp. 1309-1312]. VEGF is a  
dimer with an apparent molecular mass of about 46 kDa  
with each subunit having an apparent molecular mass of  
about 23 kDa.

30

- 2 -

VEGF has some structural similarities to platelet derived growth factor (PDGF), which is a mitogen for connective tissue cells but not mitogenic for vascular endothelial cells from large vessels.

The membrane-bound tyrosine kinase receptor, known as FLT, was shown to be a VEGF receptor [DeVries, C. *et al.*, (1992), *Science*, 255, pp.989-991]. The FLT receptor specifically binds VEGF which induces mitogenesis. Another form of the VEGF receptor, designated KDR, is also known to bind VEGF and induce mitogenesis. The partial cDNA sequence and nearly full length protein sequence of KDR is known as well [Terman, B.I. *et al.*, (1991) *Oncogene* 6, pp. 1677-1683; Terman, B.I. *et al.*, (1992) *Biochem. Biophys. Res. Comm.* 187, pp. 1579-1586].

Persistent angiogenesis may cause or exacerbate certain diseases such as psoriasis, rheumatoid arthritis, hemangiomas, angiofibromas, diabetic retinopathy and neovascular glaucoma. An inhibitor of VEGF activity would be useful as a treatment for such diseases and other VEGF-induced pathological angiogenesis and vascular permeability conditions, such as tumor vascularization.

#### SUMMARY OF THE DISCLOSURE

A naturally-occurring FLT messenger RNA (mRNA) was identified and cloned from vascular endothelial cells. This mRNA is shown to encode most of the extracellular, or soluble, portion of the VEGF receptor, FLT. Soluble receptor molecules including

- 3 -

forms containing a C-terminal transmembrane region are also recombinantly engineered for this and other VEGF receptors. These soluble receptors, comprising  
5 truncated and modified forms are expressed in recombinant host cells and have VEGF binding properties. The soluble receptor proteins are useful as inhibitors of VEGF activity since they will bind  
10 available VEGF preventing it from activating its functional receptors on vascular endothelial cells and could form non-functional heterodimers with full-length membrane anchored VEGF receptors.

#### BRIEF DESCRIPTION OF THE DRAWINGS

15

Figure 1 - A schematic diagram of full length VEGF receptors (FLT and KDR), the soluble VEGF receptors (sVEGF-RI and sVEGF-RII) and the soluble receptors  
20 containing the C-terminal transmembrane region (sVEGF-RTMI and sVEGF-RTMII) are shown with the protein domains of each.

25

Figure 2 - The DNA sequence of the sVEGF-RI soluble VEGF receptor/VEGF inhibitor is shown.

30

Figure 3 - The amino acid sequence of the sVEGF-RI soluble VEGF receptor/VEGF inhibitor is shown.

Figure 4 - Demonstration that recombinant host cells express sVEGF-RI is shown by

- 4 -

the formation of high molecular weight complexes of sVEGF-RI and [ $^{125}\text{I}$ ]VEGF and separated by size exclusion chromatography.

5

Figure 5 - A 12.5% polyacrylamide electrophoretic gel is shown which demonstrates the high degree of purity obtained for sVEGF-RI.

10

Figure 6 - Cross-linked products of sVEGF-RI and [ $^{125}\text{I}$ ]VEGF are shown at about 145 kDa, and at about 245 kDa.

15

Figure 7A and 7B - Analysis of VEGF binding to sVEGF-RI (A) and corresponding Scatchard plot (B).

20

Figure 8 - Inhibition of [ $^{125}\text{I}$ ]VEGF binding to HUVECs by sVEGF-RI is demonstrated.

Figure 9 - Inhibition of VEGF-mediated mitogenesis on HUVECs is shown using sVEGF-RI.

25

Figure 10 - The nucleotide sequence encoding sVEGF-RII is shown.

30

Figure 11 - The amino acid sequence for sVEGF-RII is shown.

- 5 -

Figure 12 - The nucleotide sequence encoding sVEGF-RTMII is shown.

5 Figure 13 - The amino acid sequence for sVEGF-RTMII is shown.

Figure 14 - The nucleotide sequence encoding sVEGF-RTMI is shown.

10 Figure 15 - The amino acid sequence for sVEGF-RTMI is shown.

Figure 16 - A diagram of pmFLT is shown.

15 Figure 17 - A diagram of pKDRA is shown.

#### DETAILED DESCRIPTION OF THE DISCLOSURE

The present invention relates to cDNA  
20 encoding a soluble VEGF receptor protein (sVEGF-R)  
which is isolated from VEGF receptor producing cells or  
is recombinantly engineered from VEGF receptor-encoding  
DNA. sVEGF-R, as used herein, refers to a protein  
which can specifically bind to a vascular endothelial  
25 cell growth factor without stimulating mitogenesis of  
vascular endothelial cells.

The amino acid sequence of FLT is known,  
[Shibuya, M. *et al.*, (1990), *Oncogene*, 5, pp.519-524]  
and corresponds to the full length cell-associated VEGF  
30 tyrosine kinase receptor. Other VEGF receptors are  
known to exist. Other known VEGF receptors include,

- 6 -

but are not limited to KDR [Terman (1991), supra., and Terman (1992), supra.]. Mammalian cells capable of producing FLT, KDR and other VEGF receptors include, but are not limited to, vascular endothelial cells. Mammalian cell lines which produce FLT or KDR and other VEGF receptors include, but are not limited to, human endothelial cells. The preferred cells for the present invention include human umbilical vein endothelial cells (HUVEC).

Other cells and cell lines may also be suitable for use to isolate sVEGF-R cDNA. Selection of suitable cells may be done by screening for sVEGF-R binding activity on cell surfaces, in cell extracts or conditioned medium or by screening for gene expression by PCR or hybridization. Methods for detecting soluble receptor activity are well known in the art [Duan, D-S. R. *et al.*, (1991) J.Biol.Chem., 266, pp.413-418] and measure the binding of labelled VEGF. Cells which possess VEGF binding activity in this assay may be suitable for the isolation of sVEGF-R cDNA.

Full length FLT producing cells such as human HUVEC cells (American Type Culture Collection, ATCC CRL 1730) [Hoshi, H. and McKeehan, W.L., Proc. Natl. Acad. Sci. U.S.A., (1984) 81, pp. 6413-6417] are grown according to the recommended culture conditions of the ATCC. Full length FLT, and KDR VEGF receptors as well as extracellular region (sVEGF-RI and sVEGF-RII) and extracellular region plus transmembrane region forms (sVEGF-RTMI and sVEGF-RTMII) are shown in Figure 1. The full length receptor has an extracellular ligand



- 7 -

binding region composed of about seven immunoglobulin-like domains, a membrane spanning sequence (transmembrane domain) and intracellular tyrosine kinase domains. The inhibitory forms of this receptor, which are the subject of the present invention, are also shown in Figure 1 and lack the intracellular kinase domains, and for some inhibitors, the transmembrane sequence and the C-terminal most Ig-like extracellular domain.

Any of a variety of procedures may be used to molecularly clone sVEGF-R cDNA. These methods include, but are not limited to, direct functional expression of the sVEGF-R gene following the construction of an sVEGF-R-containing cDNA library in an appropriate expression vector system.

Another method is to screen a sVEGF-R-containing cDNA library constructed in a bacteriophage or plasmid shuttle vector with a labelled oligonucleotide probe designed from the predicted amino acid sequence of sVEGF-R. The preferred method consists of screening a sVEGF-R-containing cDNA library constructed in a bacteriophage or plasmid shuttle vector with a partial cDNA encoding at least part of the full length FLT protein. This partial cDNA is obtained by the specific PCR amplification of sVEGF-R DNA fragments through the design of oligonucleotide primers from the known sequence of the full length FLT-encoding DNA.

It is readily apparent to those skilled in the art that other types of libraries, as well as

- 8 -

libraries constructed from other cells or cell types, may be useful for isolating sVEGF-R-encoding DNA.

Other types of libraries include, but are not limited to, cDNA libraries derived from other cells or cell lines other than HUVECs and genomic DNA libraries.

It is readily apparent to those skilled in the art that suitable cDNA libraries may be prepared from cells or cell lines which have sVEGF-R activity.

The selection of cells or cell lines for use in preparing a cDNA library to isolate sVEGF-R cDNA may be done by first measuring secreted sVEGF-R activity using the VEGF binding assay described fully herein.

Preparation of cDNA libraries can be performed by standard techniques well known in the art. Well known cDNA library construction techniques can be found for example, in Maniatis, T., Fritsch, E.F., Sambrook, J., Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1982).

It is also readily apparent to those skilled in the art that DNA encoding sVEGF-R may also be isolated from a suitable genomic DNA library.

Construction of genomic DNA libraries can be performed by standard techniques well known in the art. Well known genomic DNA library construction techniques can be found in Maniatis, T., Fritsch, E.F., Sambrook, J. in Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1982).

Another means of obtaining sVEGF-R molecules is to recombinantly engineer them from DNA encoding the

- 9 -

partial or complete amino acid sequence of a VEGF receptor. Examples of other VEGF receptors include, but are not limited to, KDR. Using recombinant DNA techniques, DNA molecules are constructed which encode at least a portion of the VEGF receptor capable of binding VEGF without stimulating mitogenesis. Standard recombinant DNA techniques are used such as those found in Maniatis, *et al.*, *supra*.

Using one of the preferred methods of the present invention, cDNA clones encoding sVEGF-R are isolated in a two-stage approach employing polymerase chain reaction (PCR) based technology and cDNA library screening. In the first stage, DNA oligonucleotides derived from the extracellular domain sequence information from the known full length FLT, KDR or other VEGF receptor is used to design degenerate oligonucleotide primers for the amplification of sVEGF-R-specific DNA fragments. In the second stage, these fragments are cloned to serve as probes for the isolation of complete sVEGF-R cDNA from a commercially available lambda gt10 cDNA library (Clontech) derived from HUVEC cells (ATCC CRL 1730).

These PCR derived products were used as hybridization probes for screening a lambda gt10 cDNA library derived from HUVECs (Clontech). Plating and plaque lifts of the library were performed by standard methods (T. Maniatis, E.F. Fritsch, J. Sambrook, Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1982). The probes were random-primed labelled with

- 10 -

32P-dCTP to high specific activity and a separate screening of the library ( $1 \times 10^6$  plaques per screen) was conducted with each probe. The probes were  
5 added to hybridization buffer (50% formamide, 5X Denhardts, 6X SSC (1X SSC = 0.15 M NaCl, 0.015 M  $\text{Na}_3\text{citrate} \cdot 2\text{H}_2\text{O}$ , pH 7.0), 0.1% SDS, 100  $\mu\text{g/ml}$  salmon sperm DNA) at  $1 \times 10^6$  cpm/ml.

Four positively hybridizing phage were  
10 detected using the flt-specific probe. These positively hybridizing phage were observed to be less than full length flt.

Two flt cDNA clones of about 2.0 kb and 2.7 kb in length were subcloned into pGEM vectors (Promega)  
15 and bi-directionally sequenced in their entirety by the chain termination method (Sanger *et al.*, (1977) P.N.A.S. USA, 74, pp. 5463-5467,) and shown to contain a single open reading frame of about 569 amino acids. Sequence analysis demonstrated that a portion of the 5'  
20 flt coding region was missing from these clones. The remainder of the 5' end was cloned using PCR and combined with the DNA of the clones lacking the 5' end to yield a single open reading frame encoding about 687 amino acids.

25 The sequence for the cDNA encoding flt-derived sVEGF-RI is shown in Table 1, and was identified in clones 7 and 11. The deduced amino acid sequence of sVEGF-RI from the cloned cDNA is shown in Table 2. Inspection of the deduced amino acid sequence  
30 reveals the presence of a single, large open reading frame of 687 amino acids. By comparison with amino

- 11 -

acid sequence of the full length FLT VEGF receptor, 31 amino acids are encoded at the C-terminal end of the cDNA which are different from those of FLT.

5           Using another of the preferred methods of the present invention, DNA encoding sVEGF-R is constructed from a DNA sequence encoding a VEGF receptor. For purposes of illustration, DNA encoding the VEGF receptor known as KDR was utilized. Using the receptor  
10 DNA sequence, a DNA molecule is constructed which encodes the extracellular domain of the receptor, or the VEGF binding domain only and is denoted sVEGF-RII. Restriction endonuclease cleavage sites are identified within the receptor DNA and can be utilized directly to  
15 excise the extracellular-encoding portion. In addition, PCR techniques as described above may be utilized to produce the desired portion of DNA. It is readily apparent to those skilled in the art that other techniques, which are standard in the art, may be  
20 utilized to produce sVEGF-R molecules in a manner analogous to those described above. Such techniques are found, for example, in Maniatis *et al.*, *supra*.

Additional truncated forms of the VEGF receptor are constructed which contain the  
25 transmembrane region. Retention of the transmembrane may facilitate orientation of the inhibitor molecule at the target cell surface. Examples of transmembrane region containing inhibitor molecules include but are not limited to those shown in Figure 1. sVEGF-RTMI and  
30 sVEGF-RTMII, as shown in Figure 1, are FLT-related and KDR-related, respectively, transmembrane region

- 12 -

containing receptor inhibitors. Construction of transmembrane region containing molecules, such as sVEGF-RTMI and sVEGF-RTMII, is done by standard techniques known in the art including but not limited to utilizing convenient restriction endonuclease cleavage sites or PCR techniques as described herein. It is readily understood by those skilled in the art that various forms of the inhibitors of a VEGF receptor, as disclosed herein, containing only the extracellular region or containing, in addition, the transmembrane region may be constructed which have substantially the same activity.

The cloned sVEGF-R cDNA obtained through the methods described above may be recombinantly expressed by molecular cloning into an expression vector containing a suitable promoter and other appropriate transcription regulatory elements, and transferred into prokaryotic or eukaryotic host cells to produce recombinant sVEGF-R. Techniques for such manipulations are fully described in Maniatis, T. *et al.*, *supra*, and are well known in the art.

Expression vectors are defined herein as DNA sequences that are required for the transcription of cloned copies of genes and the translation of their mRNAs in an appropriate host. Such vectors can be used to express eukaryotic genes in a variety of hosts such as bacteria, bluegreen algae, fungal cells, yeast cells, plant cells, insect cells and animal cells.

Specifically designed vectors allow the shuttling of DNA between hosts such as bacteria-yeast

- 13 -

or bacteria-animal or bacteria-insect cells. An appropriately constructed expression vector should contain: an origin of replication for autonomous  
5 replication in host cells, selectable markers, a limited number of useful restriction enzyme sites, a potential for high copy number, and active promoters. A promoter is defined as a DNA sequence that directs RNA polymerase to bind to DNA and initiate RNA  
10 synthesis. A strong promoter is one which causes mRNAs to be initiated at high frequency. Expression vectors may include, but are not limited to, cloning vectors, modified cloning vectors, specifically designed plasmids or viruses.

15 A variety of mammalian expression vectors may be used to express recombinant sVEGF-R in mammalian cells. Commercially available mammalian expression vectors which may be suitable for recombinant sVEGF-R expression, include but are not limited to, pMC1neo  
20 (Stratagene), pXT1 (Stratagene), pSG5 (Stratagene), EBO-pSV2-neo (ATCC 37593) pBPV-1(8-2) (ATCC 37110), pdBPV-MMTneo(342-12) (ATCC 37224), pRSVgpt (ATCC 37199), pRSVneo (ATCC 37198), pSV2-dhfr (ATCC 37146), pUCTag (ATCC 37460), and gZD35 (ATCC 37565).

25 DNA encoding sVEGF-R may also be cloned into an expression vector for expression in a recombinant host cell. Recombinant host cells may be prokaryotic or eukaryotic, including but not limited to bacteria, yeast, mammalian cells including but not limited to  
30 cell lines of human, bovine, porcine, monkey and rodent origin, and insect cells including but not limited to

- 14 -

drosophila, moth, mosquito and armyworm derived cell lines. Cell lines derived from mammalian species which may be suitable and which are commercially available, include but are not limited to, CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C127I (ATCC CRL 1616), BS-C-1 (ATCC CCL 26) and MRC-5 (ATCC CCL 171). Insect cell lines which may be suitable and are commercially available include but are not limited to 3M-S (ATCC CRL 8851) moth (ATCC CCL 80) mosquito (ATCC CCL 194 and 195; ATCC CRL 1660 and 1591) and armyworm (Sf9, ATCC CRL 1711).

The expression vector may be introduced into host cells via any one of a number of techniques including but not limited to transformation, transfection, liposome or protoplast fusion, and electroporation. The expression vector-containing cells are clonally propagated and individually analyzed to determine whether they produce sVEGF-R protein. Identification of sVEGF-R expressing host cell clones may be done by several means, including but not limited to immunological reactivity with anti-sVEGF-R antibodies, binding to radiolabelled VEGF, and the presence of host cell-secreted sVEGF-R activity.

Expression of sVEGF-R DNA may also be performed using *in vitro* produced synthetic mRNA. Synthetic mRNA can be efficiently translated in various cell-free systems, including but not limited to wheat germ extracts and reticulocyte extracts, as well as



- 15 -

efficiently translated in cell based systems, including but not limited to microinjection into frog oocytes, with microinjection into frog oocytes being preferred.

5           Levels of sVEGF-R protein produced by host cells may be quantitated by immunoaffinity and/or ligand affinity techniques. sVEGF-R-specific affinity beads or sVEGF-R-specific antibodies are used to isolate <sup>35</sup>S-methionine labelled or unlabelled sVEGF-R  
10 protein. Labelled sVEGF-R protein is analyzed by SDS-PAGE. Unlabelled sVEGF-R protein is detected by Western blotting, ELISA or RIA assays employing sVEGF-R specific antibodies, or by ligand blotting with labelled VEGF.

15           Following expression of sVEGF-R in a recombinant host cell, sVEGF-R protein may be recovered to provide sVEGF-R in active form, capable of binding VEGF without stimulating mitogenesis. Several sVEGF-R purification procedures are available and suitable for  
20 use. sVEGF-R may be purified from cell lysates and extracts, or from conditioned culture medium, by various combinations of, or individual application of salt fractionation, ion exchange chromatography, size exclusion chromatography, hydroxylapatite adsorption  
25 chromatography, reversed phase chromatography, heparin sepharose chromatography, VEGF ligand affinity chromatography, and hydrophobic interaction chromatography.

          In addition, recombinant sVEGF-R can be  
30 separated from other cellular proteins by use of an immuno-affinity column made with monoclonal or

- 16 -

polyclonal antibodies specific for full length sVEGF-R, or polypeptide fragments of sVEGF-R.

- 5    Identification of sVEGF-RI - In an attempt to clone the VEGF receptor cDNA (flt) a HUVEC  $\lambda$ gt10 cDNA library was screened with a DNA probe derived from the extracellular domain of the membrane bound or full length form of this receptor as shown in Figure 1.
- 10   Four incomplete clones, all lacking various lengths of 5' coding sequence, were isolated from screening a total of  $1 \times 10^6$  plaques. Two of these isolates represent partial clones that were identical to full length flt, one of which contained the complete 3'
- 15   coding region of the form described by Shibuya *et al.*, supra. The other two clones were identical to full length flt up to base pair number 2219 (Table 1 and Figure 2) where they then diverged from full length flt. These clones (clone 7 and 11) coded for an
- 20   additional unique 31 amino acids before the open reading frame is terminated by a TAA codon (Table 2 and Figure 3).

Clone 7 and 11 coded for a protein with a predicted molecular mass of about 75 kDa containing 12

25   putative N-linked glycosylation sites. This version of the receptor was missing the transmembrane and intracellular kinase domains and thus coded for a natural soluble form of the VEGF receptor (sVEGF-RI). Further, the protein molecule predicted by sVEGF-RI has

30   only the first six Ig-like domains, missing the one closest to the transmembrane sequence (Figure 1). The

- 17 -

31 amino acids at the C-terminal end of sVEGF-RI contain two cysteine residues, but does not resemble an Ig domain.

5

Expression of sVEGF-RI in Sf9 cells - To analyze the binding and biological properties of this form of the receptor, the protein was expressed using a baculovirus expression system. Clone 7 was missing about 350 base pairs of coding sequence at the 5' end. This region was cloned by PCR using the primers described above and in Example 1. A clone containing the complete coding region of sVEGF-RI was constructed by combining the 5' PCR fragment with sVEGF-RI clone 7 which overlapped at a SacI site. The 5' EcoRI site was then changed to a BamHI site and the full length sVEGF-RI was cloned into pBluebac III (Invitrogen) as a BamHI/BamHI fragment. A recombinant baculovirus P-3 stock containing the sVEGF-RI gene 3' in relation to the polyhedrin promoter was then prepared as described herein.

Culture media from small scale infections were tested for the ability to form high molecular weight complexes with [<sup>125</sup>I]VEGF. The labeled ligand and culture media from the baculovirus infected cells were combined and incubated. The reactions were then analyzed by size exclusion chromatography. When the wild-type infected culture medium was mixed with the radioactive ligand (Figure 4) a single radioactive peak was observed. However, when the sVEGF-RI infected culture medium was used, a high molecular weight complex was formed, as evident by the appearance of a

- 18 -

second peak in this reaction eluting near the void volume of the column. This experiment showed that the natural soluble form of the FLT VEGF receptor, sVEGF-RI, forms a high molecular weight complex with VEGF.

The recombinantly produced sVEGF-R is purified from the recombinant host cell extracts or cell culture fluid using heparin-sepharose column chromatography which specifically binds the sVEGF-R protein. The heparin-sepharose bound VEGF-R column is washed using a suitable buffer containing between 0.1M and 0.6M NaCl which removes contaminating proteins without significant loss of sVEGF-R. The sVEGF-R is eluted from the heparin-sepharose column using a suitable buffer containing about 1M NaCl, yielding substantially purified sVEGF-R.

Binding of the sVEGF-RI to VEGF - The binding of <sup>125</sup>I-labelled VEGF to sVEGF-RI was characterized by crosslinking, and by complex formation with sVEGF-RI absorbed to 96 well plates.

The crosslinked products are shown in Figure 6. The sVEGF-RI was cross-linked to [<sup>125</sup>I]VEGF (lane 1); in the presence of unlabelled VEGF (lane 2) and unlabelled bFGF (lane 3). Two high molecular weight bands (about 145 kDa and 245 kDa) were formed in the sVEGF-RI and [<sup>125</sup>I]VEGF containing reaction, and in the sVEGF-RI and [<sup>125</sup>I]VEGF plus an excess of unlabelled bFGF reaction. The two high molecular weight bands were not present when sVEGF-RI was

- 19 -

incubated with [ $^{125}$ I]VEGF plus an excess of unlabelled VEGF, demonstrating the specificity of sVEGF-RI for VEGF, and the ability of sVEGF-RI to form a dimer. The  
5 145 kDa band is presumably a crosslinked complex containing one receptor molecule (about 100 kDa) and a VEGF dimer (about 46 kDa). As shown in Figure 6 complexes containing two receptor molecules (about 245  
10 kDa) were also observed. This suggests that each VEGF dimer can bind one or two receptor molecules and that the soluble form of the VEGF receptor may undergo ligand-induced dimerization.

The affinity of sVEGF-RI for VEGF was evaluated by absorbing sVEGF-RI to the surface of a 96  
15 well plate, followed by blocking the nonspecific sites with 0.5% gelatin. Variable amounts of labeled ligand were added to each well. These results demonstrate that sVEGF-RI binds VEGF with high affinity with an apparent  $K_d$  of about 20pM (Figure 7). Since the  
20 soluble form of the receptor is missing the Ig domain closest to the transmembrane spanning region, this domain is not required for ligand binding.

The sVEGF-RI is shown to inhibit binding of VEGF to HUVECs by incubating cultured HUVECs with  
25 [ $^{125}$ I]VEGF and various amounts of sVEGF-RI. Following incubation, the cells are washed to remove unbound [ $^{125}$ I]VEGF. The cells are then solubilized and the amount of cell-associated  $^{125}$ I is determined by gamma  
30 counter, which demonstrates the amount of [ $^{125}$ I]VEGF which was capable of binding to the cellular VEGF receptor in the presence of sVEGF-RI. Using this

- 20 -

method, it is demonstrated that sVEGF-RI was capable of inhibiting [ $^{125}$ I]VEGF binding to HUVECs VEGF receptor (see Figure 8).

5        Since sVEGF-RI was able to inhibit VEGF binding to cell receptors, it was then determined that sVEGF-RI could inhibit VEGF induced mitogenesis. Cells are preincubated with sVEGF-RI and then incubated with VEGF in the presence of [ $^3$ H]thymidine. Following  
10 incubation, the amount of cellular DNA-incorporated [ $^3$ H]thymidine is measured which indicates whether VEGF has induced mitogenesis and caused [ $^3$ H]thymidine to be incorporated into cellular DNA. The presence of sVEGF-RI inhibits the ability of VEGF to stimulate  
15 mitogenesis as shown in Figure 9.

The inhibitor of the present invention can be used for the inhibition of VEGF activity. The inhibitor can be used either topically or intravascularly. For topical applications the  
20 formulation would be applied directly at a rate of about 10 ng to about 1 mg/cm<sup>2</sup>/day. For intravaneous applications, the inhibitor is used at a rate of about 1 µg to about 10 mg/kg/day of body weight. For internal use, the formulation may be released directly  
25 into the region to be treated either from implanted slow release polymeric material or from slow release pumps or repeated injections. The release rate in either case is about 100 ng to about 100 µg/day/cm<sup>3</sup>.

For non-topical application the VEGF  
30 inhibitor is administered in combination with pharmaceutically acceptable carriers or diluents such

- 21 -

as phosphate buffer, saline, phosphate buffered saline, Ringer's solution, and the like, in a pharmaceutical composition, according to standard pharmaceutical practice. For topical application, various pharmaceutical formulations are useful for the administration of the active compound of this invention. Such formulations include, but are not limited to, the following: ointments such as hydrophilic petrolatum or polyethylene glycol ointment; pastes which may contain gums such as xanthan gum; solutions such as alcoholic or aqueous solutions; gels such as aluminum hydroxide or sodium alginate gels; albumins such as human or animal albumins; collagens such as human or animal collagens; celluloses such as alkyl celluloses, hydroxy alkyl celluloses and alkylhydroxyalkyl celluloses, for example methylcellulose, hydroxyethyl cellulose, carboxymethyl cellulose, hydroxypropyl methylcellulose, and hydroxypropyl cellulose; polyoxamers such as Pluronic® Polyols exemplified by Pluronic® F-127; tetronics such as tetronic 1508; and alginates such as sodium alginate.

The following examples are provided as illustrative of the present invention without, however, limiting the same thereto.

#### EXAMPLE 1

Cloning flt-related sVEGF-RI - A 580 base pair DNA probe for flt was obtained by PCR of the HUVEC phage library using the primers 5' GCACCTTGTTGTGGCTGAC 3'

- 22 -

(SEQ. ID. No.: 1) and 5' TGGAATTCGTGCTGCTTCCTGGTCC  
3' (SEQ. ID. No.: 2). The resulting DNA fragment was  
cloned into pGEM3Z as a XbaI/EcoRI fragment. The  
5 probe was prepared by the random priming method  
[Feinberg, A.P. and Vogelstein, B., (1983)  
Anal.Biochem., 132, pp.6-13] using the megaprime kit  
(Amersham) at a specific activity of  $1 \times 10^7$  cpm/ng.  
The HUVEC cDNA library was plated at a density of  $5 \times$   
10  $10^4$  plaques/150 cm plate then about  $1 \times 10^6$  plaques  
were screened by hybridization as previously described  
[Maniatis, T. *et al.*, supra]. Briefly, following  
prehybridization at 42°C for 2 hours in 50% formamide,  
5X SSC, 5X Denhardt's solution, 0.1% SDS, 100 µg/ml  
15 salmon sperm DNA (hybridization buffer) the filters  
were hybridized with the probe for 16 hours at 42°C in  
hybridization buffer. The filters were washed one  
time for 15 min at room temperature in 2X SSC then  
three times at 55°C in 0.1 X SSC. Four positive  
20 plaques were identified and rescreened two additional  
times to obtain homogeneous isolates. Inserts were  
cloned into pGEM3Z for DNA sequence analysis. Two of  
these clones were identified which contained less than  
the full length flt coding region. DNA sequence  
25 analysis showed that these clones lacked the 5' coding  
region of flt. The DNA sequence is shown in Table 1  
and Figure 2, and the deduced amino acid sequence is  
shown in Table 2 and Figure 3. The 5' end of flt was  
cloned by PCR using the primers 5'  
30 GGAATTCGCGCTCACCATGGTCAGC 3' (SEQ.ID.NO.:3) and 5'  
TTTGAATTCACCCGGCAGGGAATGACG 3' (SEQ.ID.NO.:4). The  
PCR fragment generated with this set of primers was  
cloned into flt clone 7 as an EcoRI/SacI fragment.



- 23 -

TABLE 1

5 GCGGACACTCTCTCGGCTCCTCCCCGGCAGCGGCGGCGGCTCGGAGCGGGCTCCGGGG  
CTCGGGTGCAGCGGCCAGCGGCCTGCGGGCGAGGATTACCGGGGAAGTGGTTGTCTC  
CTGGCTGGAGCCCGCAGACGGCCCTCAGGGCGGGGGCCGGCGGGCGGAACGAGAGG  
10 ACGGACTCTGGCGGCCGGGTGCTTGGCCGGGGAGCGCGGCACCGGGCGAGCAGGCCG  
CGTCGCGCTCACC ATG GTC AGC TAC TGG GAC ACC GGG GTC CTG CTG  
TGC GCG CTG CTC AGC TGT CTG CTT CTC ACA GGA TCT AGT TCA GGT  
15 TCA AAA TTA AAA GAT CCT GAA CTG AGT TTA AAA GGC ACC CAG CAC  
ATC ATG CAA GCA GGC CAG ACA CTG CAT CTC CAA TGC AGG GGG GAA  
20 GCA GCC CAT AAA TGG TCT TTG CCT GAA ATG CTC AGT AAG GAA ACC  
GAA AGG CTG AGC ATA ACT AAA TCT GCC TGT GGA AGA AAT GGC AAA

25

30

- 24 -

CAA TTC TGC AGT ACT TTA ACC TTG AAC ACA GCT CAA GCA AAC CAC  
ACT GGC TTC TAC AGC TGC AAA TAT CTA GCT GTA CCT ACT TCA AAG  
5 AAG AAG GAA ACA GAA TCT GCA ATC TAT ATA TTT ATT AGT GAT ACA  
GGT AGA CCT TTC GTA GAG ATG TAC AGT GAA ATC CCC GAA ATT ATA  
10 CAC ATG ACT GAA GGA AGG GAG CTC GTC ATT CCC TGC CGG GTT ACG  
TCA CCT AAC ATC ACT GTT ACT TTA AAA AAG TTT CCA CTT GAC ACT  
TTG ATC CCT GAT GGA AAA CGC ATA ATC TGG GAC AGT AGA AAG GGC  
15 TTC ATC ATA TCA AAT GCA ACG TAC AAA GAA ATA GGG CTT CTG ACC  
TGT GAA GCA ACA GTC AAT GGG CAT TTG TAT AAG ACA AAC TAT CTC  
20 ACA CAT CGA CAA ACC AAT ACA ATC ATA GAT GTC CAA ATA AGC ACA

25

30

- 25 -

CCA CGC CCA GTC AAA TTA CTT AGA GGC CAT ACT CTT GTC CTC AAT  
TGT ACT GCT ACC ACT CCC TTG AAC ACG AGA GTT CAA ATG ACC TGG  
5 AGT TAC CCT GAT GAA AAA AAT AAG AGA GCT TCC GTA AGG CGA CGA  
ATT GAC CAA AGC AAT TCC CAT GCC AAC ATA TTC TAC AGT GTT CTT  
10 ACT ATT GAC AAA ATG CAG AAC AAA GAC AAA GGA CTT TAT ACT TGT  
CGT GTA AGG AGT GGA CCA TCA TTC AAA TCT GTT AAC ACC TCA GTG  
CAT ATA TAT GAT AAA GCA TTC ATC ACT GTG AAA CAT CGA AAA CAG  
15 CAG GTG CTT GAA ACC GTA GCT GGC AAG CGG TCT TAC CGG CTC TCT  
ATG AAA GTG AAG GCA TTT CCC TCG CCG GAA GTT GTA TGG TTA AAA  
20 GAT GGG TTA CCT GCG ACT GAG AAA TCT GCT CGC TAT TTG ACT CGT

25

30

- 26 -

GGC TAC TCG TTA ATT ATC AAG GAC GTA ACT GAA GAG GAT GCA GGG  
AAT TAT ACA ATC TTG CTG AGC ATA AAA CAG TCA AAT GTG TTT AAA  
5 AAC CTC ACT GCC ACT CTA ATT GTC AAT CTG AAA CCC CAG ATT TAC  
GAA AAG GCC GTG TCA TCG TTT CCA GAC CCG GCT CTC TAC CCA CTG  
10 GGC AGC AGA CAA ATC CTG ACT TGT ACC GCA TAT GGT ATC CCT CAA  
CCT ACA ATC AAG TGG TTC TGG CAC CCC TGT AAC CAT AAT CAT TCC  
GAA GCA AGG TGT GAC TTT TGT TCC AAT AAT GAA GAG TCC TTT ATC  
15 CTG GAT GCT GAC AGC AAC ATG GGA AAC AGA ATT GAG AGC ATC ACT  
CAG CGC ATG GCA ATA ATA GAA GGA AAG AAT AAG ATG GCT AGC ACC  
20 TTG GTT GTG GCT GAC TCT AGA ATT TCT GGA ATC TAC ATT TGC ATA

25

30

- 27 -

GCT TCC AAT AAA GTT GGG ACT GTG GGA AGA AAC ATA AGC TTT TAT  
ATC ACA GAT GTG CCA AAT GGG TTT CAT GTT AAC TTG GAA AAA ATG  
5 CCG ACG GAA GGA GAG GAC CTG AAA CTG TCT TGC ACA GTT AAC AAG  
TTC TTA TAC AGA GAC GTT ACT TGG ATT TTA CTG CGG ACA GTT AAT  
10 AAC AGA ACA ATG CAC TAC AGT ATT AGC AAG CAA AAA ATG GCC ATC  
ACT AAG GAG CAC TCC ATC ACT CTT AAT CTT ACC ATC ATG AAT GTT  
TCC CTG CAA GAT TCA GGC ACC TAT GCC TGC AGA GCC AGG AAT GTA  
15 TAC ACA GGG GAA GAA ATC CTC CAG AAG AAA GAA ATT ACA ATC AGA  
GGT GAG CAC TGC AAC AAA AAG GCT GTT TTC TCT CGG ATC TCC AAA  
20 TTT AAA AGC ACA AGG AAT GAT TGT ACC ACA CAA AGT AAT GTA AAA  
CAT TAA

25

30

- 28 -

AGGACTCATTAAAAAGTAACAGTTGTCTCATATCATCTTGATTTATTGTCACTGTTG

CTAACTTTCAGGCTCGGAGGAGATGCTCCTCCCAAATGAGTTCGGAGATGATAGCA

5

GTAATAATGAGACCCCGGGCTCCAGCTCTGGGCCCCCATTGAGCCGAGGGGGCT

GCTCCGGGGGGCCGACTTGCTGCACGTTTGGATTTGGAGGATCCCTGCACTGCCTTC

10 TCTGTGTTTGTGCTCTTGCTGTTTTCTCCTGCCTGATAACAACAACCTGGGATGA

TCCTTTCATTTTGATGCCAACCTCTTTTATTTTAAGCGGGCCCTATAGT

(SEQ. ID. NO.: 5)

15

20

25

30

- 29 -

TABLE 2

5 Met Val Ser Tyr Trp Asp Thr Gly Val Leu Leu  
Cys Ala Leu Leu Ser Cys Leu Leu Leu Thr Gly Ser Ser Ser Gly  
Ser Lys Leu Lys Asp Pro Glu Leu Ser Leu Lys Gly Thr Gln His  
10 Ile Met Gln Ala Gly Gln Thr Leu His Leu Gln Cys Arg Gly Glu  
Ala Ala His Lys Trp Ser Leu Pro Glu Met Val Ser Lys Glu Ser  
Glu Arg Leu Ser Ile Thr Lys Ser Ala Cys Gly Arg Asn Gly Lys  
15 Gln Phe Cys Ser Thr Leu Thr Leu Asn Thr Ala Gln Ala Asn His  
Thr Gly Phe Tyr Ser Cys Lys Tyr Leu Ala Val Pro Thr Ser Lys  
20 Lys Lys Glu Thr Glu Ser Ala Ile Tyr Ile Phe Ile Ser Asp Thr  
Gly Arg Pro Phe Val Glu Met Tyr Ser Glu Ile Pro Glu Ile Ile

25

30

- 30 -

His Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val Thr  
Ser Pro Asn Ile Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr  
5 Leu Ile Pro Asp Gly Lys Arg Ile Ile Trp Asp Ser Arg Lys Gly  
Phe Ile Ile Ser Asn Ala Thr Tyr Lys Glu Ile Gly Leu Leu Thr  
10 Cys Glu Ala Thr Val Asn Gly His Leu Tyr Lys Thr Asn Tyr Leu  
Thr His Arg Gln Thr Asn Thr Ile Ile Asp Val Gln Ile Ser Thr  
Pro Arg Pro Val Lys Leu Leu Arg Gly His Thr Leu Val Leu Asn  
15 Cys Thr Ala Thr Thr Pro Leu Asn Thr Arg Val Gln Met Thr Trp  
Ser Tyr Pro Asp Glu Lys Asn Lys Arg Ala Ser Val Arg Arg Arg  
20 Ile Asp Gln Ser Asn Ser His Ala Asn Ile Phe Tyr Ser Val Leu

25

30



- 31 -

Thr Ile Asp Lys Met Gln Asn Lys Asp Lys Gly Leu Tyr Thr Cys  
Arg Val Arg Ser Gly Pro Ser Phe Lys Ser Val Asn Thr Ser Val  
5 His Ile Tyr Asp Lys Ala Phe Ile Thr Val Lys His Arg Lys Gln  
Gln Val Leu Glu Thr Val Ala Gly Lys Arg Ser Tyr Arg Leu Ser  
10 Met Lys Val Lys Ala Phe Pro Ser Pro Glu Val Val Trp Leu Lys  
Asp Gly Leu Pro Ala Thr Glu Lys Ser Ala Arg Tyr Leu Thr Arg  
Gly Tyr Ser Leu Ile Ile Lys Asp Val Thr Glu Glu Asp Ala Gly  
15 Asn Tyr Thr Ile Leu Leu Ser Ile Lys Gln Ser Asn Val Phe Lys  
Asn Leu Thr Ala Thr Leu Ile Val Asn Val Lys Pro Gln Ile Tyr  
20 Glu Lys Ala Val Ser Ser Phe Pro Asp Pro Ala Leu Tyr Pro Leu

25

30

- 32 -

Gly Ser Arg Gln Ile Leu Thr Cys Thr Ala Tyr Gly Ile Pro Gln  
Pro Thr Ile Lys Trp Phe Trp His Pro Cys Asn His Asn His Ser  
5 Glu Ala Arg Cys Asp Phe Cys Ser Asn Asn Glu Glu Ser Phe Ile  
Leu Asp Ala Asp Ser Asn Met Gly Asn Arg Ile Glu Ser Ile Thr  
10 Gln Arg Met Ala Ile Ile Glu Gly Lys Asn Lys Met Ala Ser Thr  
Leu Val Val Ala Asp Ser Arg Ile Ser Gly Ile Tyr Ile Cys Ile  
Ala Ser Asn Lys Val Gly Thr Val Gly Arg Asn Ile Ser Phe Tyr  
15 Ile Thr Asp Val Pro Asn Gly Phe His Val Asn Leu Glu Lys Met  
Pro Thr Glu Gly Glu Asp Leu Lys Leu Ser Cys Thr Val Asn Lys  
20 Phe Leu Tyr Arg Asp Val Thr Trp Ile Leu Leu Arg Thr Val Asn

25

30

- 33 -

Asn Arg Thr Met His Tyr Ser Ile Ser Lys Gln Lys Met Ala Ile  
Thr Lys Glu His Ser Ile Thr Leu Asn Leu Thr Ile Met Asn Val  
5 Ser Leu Gln Asp Ser Gly Thr Tyr Ala Cys Arg Ala Arg Asn Val  
Tyr Thr Gly Glu Glu Ile Leu Gln Lys Lys Glu Ile Thr Ile Arg  
10 Gly Glu His Cys Asn Lys Lys Ala Val Phe Ser Arg Ile Ser Lys  
Phe Lys Ser Thr Arg Asn Asp Cys Thr Thr Gln Ser Asn Val Lys  
His \*\*\* (SEQ. ID. NO.: 6)  
15

#### EXAMPLE 2

Expression of sVEGF-RI in Sf9 insect cells - The full  
20 length sequence encoding sVEGF-RI was cloned as an  
EcoRI/BamHI fragment into pGEM3Z. The EcoRI site was  
then modified to a BamHI site and cloned into pBlueBac  
III 3' of the polyhedrin promoter (psFLTblue). This  
plasmid was transfected into Sf9 armyworm cells using  
25 liposomes. After 48 hours the medium from the  
transfected cells which contains recombinant polyhedrin  
virus particles, was harvested. Dilutions ( $10^3$  -  $10^4$   
fold) of the virus were prepared and plaque purified in  
soft agar containing 150 µg/ml 5-bromo-4-chloro-3-  
30

- 34 -

indolyl- $\beta$ -D-galactoside. Recombinant plaques were identified by blue color and used to infect Sf9 cells ( $5 \times 10^5$  cells/well) in 12 well plates. Medium (100  $\mu$ l) from polyhedrin minus infections was used to prepare P-2 viral stocks by infecting  $2.5 \times 10^6$  cells in a T-25 flask. Large scale high titer P-3 viral stocks were then prepared by infecting Sf9 cells (500 ml at  $2 \times 10^6$  cells/ml) with 5 ml of the P-2 stock then incubating at 27°C for 5 - 6 days and the medium was harvested by centrifugation. Protein expression was accomplished by infecting cells at a density of  $2-2.5 \times 10^6$  cells/ml with a multiplicity of infection of 5 - 10. Twenty four hours after infection the cells were changed to a serum free medium (SF900II, Gibco BRL), incubated for an additional 48 hours and the medium was collected. This conditioned medium contains the recombinantly expressed sVEGF-RI protein.

20

EXAMPLE 3

Iodination of VEGF -  $^{125}\text{I}$ -labeled human recombinant VEGF was prepared by the chloramine T method (Hunter, W.M. and Greenwood, F.C., (1962) Nature (London), 194, pp. 495-496). Briefly, 1  $\mu$ g of VEGF in 30% acetonitrile/0.1% trifluoroacetic acid was adjusted to pH 7.1 by the addition of 1/3 volume of 0.4 M sodium phosphate buffer, pH 7.1. Freshly dissolved chloramine T (4  $\mu$ l of a 2 mg/ml stock in 0.1 M sodium phosphate buffer, pH 7.1) was added to the VEGF solution and reacted for 45 seconds at room temperature (total

- 35 -

volume of 150  $\mu$ l). The reaction was stopped by the addition of 50  $\mu$ l of 10 mM KI and 50  $\mu$ l of 2 mg/ml meta bisufite. The labeled ligand was separated from the free  $^{125}\text{I}$  by gel filtration on a 0.7 X 15 cm Sephadex G-25 column equilibrated in PBS with 1 mg/ml gelatin. Fractions were counted in a Packard  $\gamma$  counter, aliquoted and stored at  $-70^{\circ}\text{C}$ . VEGF was labeled to a specific activity of  $5 \times 10^5$  to  $1 \times 10^6$  cpm/ng.

Gel Filtration Chromatography - Receptor-ligand complex was formed by incubating 10  $\mu$ l of  $^{125}\text{I}$ -labeled VEGF ( $10^5$  cpm) with 100  $\mu$ l of either wild-type or baculovirus sVEGF-RI-containing, infected Sf9 cell culture medium overnight at room temperature. The reaction products were separated on a Sephacryl S200 gel filtration column (0.7 X 25 cm) equilibrated in PBS, 1 mg/ml gelatin, at a flow rate of 15 ml/hr. Fractions (0.75 ml) were collected and analyzed in a  $\gamma$  counter. Receptor-ligand complexes pass quickly through the column while the free labelled VEGF passes through more slowly. The results of this experiment shown in Figure 4 demonstrate the formation of a high molecular weight complex between labelled VEGF and sVEGF-RI protein. This shows that sVEGF-RI binds VEGF.

Crosslinking - Purified sVEGF-RI (1-10ng) was added to 25  $\mu$ l of binding buffer (Dulbecco's Modified Eagle's medium (DME), 25 mM HEPES, pH 7.5, 0.3% gelatin), and  $1 \times 10^5$  cpm of [ $^{125}\text{I}$ ]-VEGF was added (Figure 6, lane 1) with either 200ng of unlabelled VEGF (lane 2) or bFGF

- 36 -

(lane 3), then incubated 2 to 16 hours at room temperature. Bis(sulfosuccinimidyl)suberate (Pierce) crosslinker was added to a final concentration of 1 mM. The reaction was stopped after 15 min by the addition of boiling SDS PAGE sample buffer. The crosslinked products were separated by SDS PAGE on a 7.5% acrylamide gel and analyzed either by autoradiography or a phosphoimager. The results are shown in Figure 6 and demonstrate that sVEGF-RI binds labelled VEGF by the appearance of two bands of about 145 kDa and 245 kDa. The 145 kDa band consists of one sVEGF-RI molecule and one VEGF molecule (Monomer, M.). The 245 kDa band apparently consists of two sVEGF-RI molecules and one VEGF dimer (D). Free VEGF ligand (L) dimers migrated at about 45 kDa.

Binding assay - The binding of sVEGF-RI to VEGF was analyzed using a 96 well plate assay as described by Duan, D-S. R. *et al.*, supra. Briefly, sVEGF-RI, 50 to 200  $\mu$ l partially purified by Mono Q chromatography (Pharmacia), was diluted to 10 ml in 25 mM TRIS, pH 7.4, 100 mM NaCl, 20 mM  $\text{NH}_4\text{HCO}_3$ . Aliquots (100  $\mu$ l) were absorbed to the surface of a 96 well plate for 18 hours at 4°C, the plates were then washed twice with blocking buffer (DME, 25 mM HEPES, pH 7.5, 0.5% gelatin) and the nonspecific sites were blocked in the same buffer for 6 hours at 4°C. The plate was then washed twice in binding buffer. Various amounts of [ $^{125}\text{I}$ ]VEGF were added to the wells in a final volume of 100  $\mu$ l/well and incubated for 2 hours at room

- 37 -

temperature. The wells were washed three times with 100  $\mu$ l of binding buffer, the bound protein was solubilized with 100  $\mu$ l of 1% SDS, 0.5% BSA and counted in a  $\gamma$  counter. The results, shown in Figure 7, were analyzed by the method of Scatchard [Scatchard, G., (1949) Ann. N.Y. Acad. Sci., 51, pp. 660-672]. The analysis demonstrates that sVEGF-RI retains high affinity binding for VEGF with a  $K_d$  value of about 20 pM. This clearly demonstrates that sVEGF-RI, lacking the transmembrane region and adjacent Ig-like domain, binds VEGF with high affinity and that these regions are not required for VEGF binding.

15

#### EXAMPLE 4

Inhibition of VEGF binding by sVEGF-RI - The ability of sVEGF-RI to inhibit VEGF binding to HUVECs was tested. HUVECs were plated at 50,000 cells/well in 24 well plates precoated with gelatin, and allowed to grow to confluence. A constant amount of [ $^{125}$ I]VEGF (100,000 cpm) was mixed with various amounts of partially purified sVEGF-RI in binding buffer, in a total volume of 200  $\mu$ l and preincubated at room temperature for 1 hour. Samples were added to the cells and incubated for 4 hours at 4°C with shaking. The medium was then aspirated and the cells were washed three times with binding buffer. The bound radioactivity was solubilized with 50 mM TRIS-HCl, pH 8.0, 150 mM NaCl, 1% NP40, 1% BSA and counted in a  $\gamma$  counter.

- 38 -

The results are shown in Figure 8. At the highest concentration of sVEGF-RI, VEGF binding to HUVECs was reduced by 70%. It may, however, be difficult to completely inhibit binding to the cellular membrane bound receptor since one molecule of sVEGF-R bound to a VEGF dimer may be able to bind to cell associated receptor to form an inactive (sVEGF-RI)-VEGF-(membrane spanning VEGF receptor) complex.

#### EXAMPLE 5

Inhibition of VEGF mediated mitogenesis by sVEGF-RI  
Mitogenic inhibition - Since sVEGF-RI was able to inhibit VEGF binding to endothelial cells, it was then determined that the soluble receptor could inhibit VEGF induced mitogenesis in HUVECs. HUVECs were plated in gelatin coated 96 well plates at a density of 4000 cells/well in 100  $\mu$ l of DME supplemented with 10% heat inactivated fetal calf serum plus antibiotics (penicillin G, 100 units/ml; streptomycin sulfate, 100  $\mu$ g/ml). After 16 hours the medium was changed and test samples were added, cells were preincubated with a variable amount of purified sVEGF-RI for 15 minutes at 37°C before growth factor (10 ng/ml) was added. The cells were incubated for 24 hours then [methyl-<sup>3</sup>H]thymidine (0.8  $\mu$ Ci/well; 20 Ci/mmol; 1Ci = 37 GBq, final specific activity of 0.8  $\mu$ Ci/nmole) was added followed by incubated for an additional 72 hours at 37°C under 5% CO<sub>2</sub>. The cells were then washed twice with Hank's balanced salt solution adjusted to pH 7.5



- 39 -

with 25 mM Hepes, 0.1% BSA. The cells were then lysed, the DNA was solubilized with 0.2 M  $\text{Na}_2\text{CO}_3$ , 0.1 M NaOH, and [ $^3\text{H}$ ]thymidine incorporation was quantified by scintillation counting. The results are shown in Figure 9. sVEGF-RI was able to completely inhibit VEGF induced [ $^3\text{H}$ ]thymidine incorporation in HUVECs.

#### EXAMPLE 6

Purification of baculovirus expressed sVEGF-RI from Sf9 cells - Culture medium from Sf9 cells infected with a baculovirus construct designed to express sVEGF-RI (Example 2) was chromatographed through a heparin Sepharose CL-6B (Pharmacia) column (0.7 X 4 cm). The column was washed with 5 volumes of 10 mM Na-phosphate buffer, pH 6.2, 0.1 M NaCl, followed by 6 ml of 10 mM Na-phosphate buffer, pH 6.2, 0.6 M NaCl. The sVEGF-RI was eluted with 10 mM Na-phosphate buffer, pH 6.2, 1.0 M NaCl. Polyacrylamide gel electrophoresis was performed which demonstrated greater than 90% purity (as judged by coomassie blue staining) of the recombinantly produced sVEGF-R (Figure 5). The identity of the protein was confirmed by N-terminal protein sequence analysis. The actual N-terminus (Ser Lys Leu ...) of the recombinant protein differs by two amino acids from that predicted by Shibuya et al., supra. (Ser-Ser-Ser...). The peptidase cleavage site in sVEGF-RI produced in Sf9 cells was between residues gly-26 and ser-27.

- 40 -

EXAMPLE 7

Construction of KDR-related sVEGF-R - Soluble forms of  
5 KDR (a known VEGF receptor) [Terman, B.I. et al.,  
(1991) Oncogene 6, pp. 1677-1683; Terman, B.I. et al.,  
(1992) Biochem. Biophys. Res. Comm. 187, pp. 1579-1586]  
may exist naturally but have not yet been identified.  
A soluble form of KDR is recombinantly constructed by  
10 modifying its coding sequence by PCR using the primers  
1) 5' TTTTGGATCCCTGCAGACAGATCTACGTTTGAGAACC 3' (SEQ.  
ID. NO.: 7) and 2) 5' TTTTGGATCCTTAACGCTCTAGGACTGTGAGC  
3' (SEQ. ID. NO.: 8), and pKDRA (the XhoI/EcoRI  
15 fragment coding for the extracellular and transmembrane  
domain of KDR cloned into the EcoRI site of pGEM 7Z  
obtained from Promega) as a template (Figure 17). This  
generated a translation stop codon after amino acid  
residue number 663 of KDR which corresponds to the  
extracellular domain of full length KDR. This modified  
20 fragment is then used to replace the PstI/BamHI  
fragment of pKDRA generating a truncated form of the  
KDR gene (Figure 10) which codes for a soluble receptor  
denoted sVEGF-RII (Figure 11). The XhoI site at base  
pair number 257 is then changed to a BamHI site by  
25 standard cloning techniques. Another truncated form of  
the KDR receptor is created with primer 1 shown above,  
and primer 3) 5' TTTTGGATCCAACGGTCCCTAGGATGATGAC 3' ,  
(SEQ. ID. NO.: 9) (Figure 12). This form of KDR,  
denoted sVEGF-RTMII, is truncated at the C-terminal  
30 side of the transmembrane domain and therefore retains  
the transmembrane region (Figure 13). A similar form  
of the FLT receptor is generated by PCR using the

- 41 -

primers 4) 5' AGCACCTTGGTTGTGGCTGACTC 3' (SEQ. ID. NO.: 10) and 5) 5' TTTTGGATCCTTAGATAAGGAGGGTTAATAGG 3' (SEQ. ID. NO.: 11) and plasmid pmFLT (full length flt cloned into the EcoRI site of pGEM3Z obtained from Promega) as a template (Figure 16). The 780 base pair PCR fragment can then be cloned together with the EcoRI/XbaI fragment from pmFLT to produce an EcoRI/BAMHI fragment (Figure 14) encoding a truncated form of FLT (denoted sVEGF-RTMI) which retains the transmembrane domain but lacks the cytoplasmic domain (Figure 15). The EcoRI site at the 5' end of the gene is then modified to a BamHI site. The resulting truncated forms of KDR and FLT are then cloned into pBluebac111 (Stratagene) for expression in Sf9 insect cells. Characterization of these constructed truncated forms of VEGF receptors is accomplished by the techniques used to characterize sVEGF-RI as in Examples 2, 3, 4, 5, and 6.

20

25

30

- 42 -

## SEQUENCE LISTING

5

## (1) GENERAL INFORMATION:

(i) APPLICANT: Thomas, Kenneth A.  
Kendall, Richard L.

10

(ii) TITLE OF INVENTION: INHIBITOR OF VASCULAR ENDOTHELIAL CELL  
GROWTH FACTOR

15

(iii) NUMBER OF SEQUENCES: 18

## (iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Merck & Co., Inc.  
(B) STREET: P.O. Box 2000 126 E Lincoln Avenue  
(C) CITY: Rahway  
(D) STATE: NJ  
(E) COUNTRY: USA  
(F) ZIP: 07065-0907

20

## (v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

25

30

## (vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:  
(B) FILING DATE:  
(C) CLASSIFICATION:

- 43 -

## (viii) ATTORNEY/AGENT INFORMATION:

- (A) NAME: Wallen, John W.III  
(B) REGISTRATION NUMBER: 35,403  
(C) REFERENCE/DOCKET NUMBER: 18888

## (ix) TELECOMMUNICATION INFORMATION:

- (A) TELEPHONE: (908) 594-3905  
(B) TELEFAX: (908) 594-4720

## (2) INFORMATION FOR SEQ ID NO:1:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GCACCTTG6T TGTGGCTGAC

20

## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

- 44 -

(ii) MOLECULE TYPE: cDNA

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

TGGAAATTCGT GCTGCTTCCT GGTC

25

10

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26 base pairs

(B) TYPE: nucleic acid

15

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GGAATTCGCG GCTCACCATG GTCAGC

26

25

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27 base pairs

(B) TYPE: nucleic acid

30

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

- 45 -

(ii) MOLECULE TYPE: cDNA

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TTTGAATTCA CCCGGCAGGG AATGACG

27

10

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2313 base pairs

(B) TYPE: nucleic acid

15

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

25

GCGGACACTC CTCTCGGCTC CTCCCCGGCA GCGGCGGCGG CTCGGAGCGG GCTCCGGGGC 60

TCGGGTGCAG CGGCCAGCGG GCCTGGCGGC GAGGATTACC CGGGGAAGTG GTTGTCTCCT 120

GGCTGGAGCC GCGAGACGGG CGCTCAGGCG GCGGGGCCGG CGGCGGCGAA CGAGAGGACG 180

30

GACTCTGGCG GCCGGGTCGT TGGCCGGGGG AGCGCGGGCA CCGGCGGAGC AGGCCGCGTC 240

GCGCTACCA TGGTCAGCTA CTGGGACACC GGGGTCCTGC TGTGCGCGCT GCTCAGCTGT 300

- 46 -

	CTGCTTCTCA CAGGATCTAG TTCAGGTTCA AAATTTAAAG ATCCTGAACT GAGTTTAAAA	360
5	GGCACCCAGC ACATCATGCA AGCAGGCCAG AACTGCATC TCCAATGCAG GGGGGAAGCA	420
	GCCCATAAAT GGTCTTTGCC TGAAATGGTG AGTAAGGAAA GCGAAAGGCT GAGCATAACT	480
	AAATCTGCCT GTGGAAGAAA TGGCAAACAA TTCTGCAGTA CTTTAACTT GAACACAGCT	540
10	CAAGCAAACC AACTGGCTT CTACAGETGC AAATATCTAG CTGTACCTAC TTCAAAGAAG	600
	AAGGAAACAG AATCTGCAAT CTATATATT ATTAGTGATA CAGGTAGACC TTTCGTAGAG	660
15	ATGTACAGTG AAATCCCCGA AATTATACAC ATGACTGAAG GAAGGGAGCT CGTCATTCCC	720
	TGCCGGGTTA CGTCACCTAA CATCACTGTT ACTTTAAAA AGTTTCCACT TGACACTTTG	780
	ATCCCTGATG GAAAACGCAT AATCTGGGAC AGTAGAAAGG GCTTCATCAT ATCAAATGCA	840
20	ACGTACAAAG AAATAGGGCT TCTGACCTGT GAAGCAACAG TCAATGGGCA TTTGTATAAG	900
	ACAACTATC TCACACATCG ACAAACCAAT ACAATCATAG ATGTCCAAAT AAGCACACCA	960
25	CGCCCAGTCA AATTACTTAG AGGCCATACT CTTGTCTCA ATTGTACTGC TACCACTCCC	1020
	TTGAACACGA GAGTTCAAAT GACCTGGAGT TACCCTGATG AAAAAATAA GAGAGCTTCC	1080
	GTAAGGCGAC GAATTGACCA AAGCAATTCC CATGCCAACA TATTCTACAG TGTCTTACT	1140
30	ATTGACAAAA TGCAGAACAA AGACAAAGGA CTTTATACTT GTCGTGTAAG GAGTGGACCA	1200
	TCATTCAAAT CTGTTAACAC CTCAGTGCAT ATATATGATA AAGCATTCACT CACTGTGAAA	1260



- 47 -

	CATCGAAAAC AGCAGGTGCT TGA AACCGTA GCTGGCAAGC GGTCTTACC6 GCTCTCTATG	1320
5	AAAGTGAAGG CATTTCCTC GCCGGAAGTT STATGGTTAA AAGATGGGT ACCTGCGACT	1380
	GAGAAATCTG CTCGCTATT GACTCGTGC TACTCGTTAA TTATCAAGGA CGTAACTGAA	1440
	GAGGATGCAG GGAATTATAC AATCTTGCTG AGCATAAAAC AGTCAAATGT GTTTAAAAAC	1500
10	CTCACTGCCA CTCTAATTGT CAATGTGAAA CCCAGATTT ACGAAAAGGC CGTGTCACTG	1560
	TTCCAGACC CGGCTCTCTA CCCACTGGGC AGCAGACAAA TCCTGACTTG TACCGCATAT	1620
15	GGTATCCCTC AACCTACAAT CAAGTGGTTC TGGCACCCT GTAACCATAA TCATTCCGAA	1680
	GCAAGGTGTG ACTTTTGTT CAATAATGAA GAGTCCTTTA TCCTGGATGC TGACAGCAAC	1740
	ATGGGAAACA GAATTGAGAG CATCACTCAG CGCATGGCAA TAATAGAAGG AAAGAATAAG	1800
20	ATGGCTAGCA CCTTGTTGT GGCTGACTCT AGAATTTCTG GAATCTACAT TTGCATAGCT	1860
	TCCAATAAAG TTGGGACTGT GGAAGAAAC ATAAGCTTT ATATCACAGA TGTGCCAAAT	1920
25	GGGTTTCATG TTAAGTTGA AAAATGCCG ACGGAAGGAG AGGACCTGAA ACTGTCTTGC	1980
	ACAGTTAACA AGTTCTTATA CAGAGACGTT ACTTGGATTT TACTGCGGAC AGTTAATAAC	2040
	AGAACAATGC ACTACAGTAT TAGCAAGCAA AAAATGGCCA TCACTAAGGA GCACTCCATC	2100
30	ACTCTTAATC TTACCATCAT GAATGTTCC CTGCAAGATT CAGGCACCTA TGCCTGCAGA	2160
	GCCAGGAATG TATACACAGG GGAAGAAATC CTCCAGAAGA AAGAAATTAC AATCAGAGGT	2220

- 48 -

BAGCACTGCA ACAAAAAGGC TGTTTCTCT CGGATCTCCA AATTAAAAAG CACAAGGAAT 2280

GATTGTACCA CACAAAGTAA TGTAACAT TAA 2313

5

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

10

(A) LENGTH: 687 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

15

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

20

Met Val Ser Tyr Trp Asp Thr Gly Val Leu Leu Cys Ala Leu Leu Ser  
1 5 10 15

Cys Leu Leu Leu Thr Gly Ser Ser Ser Gly Ser Lys Leu Lys Asp Pro  
20 25 30

25

Glu Leu Ser Leu Lys Gly Thr Gln His Ile Met Gln Ala Gly Gln Thr  
35 40 45

30

Leu His Leu Gln Cys Arg Gly Glu Ala Ala His Lys Trp Ser Leu Pro  
50 55 60

Glu Met Val Ser Lys Glu Ser Glu Arg Leu Ser Ile Thr Lys Ser Ala  
65 70 75 80

- 49 -

	Cys Gly Arg Asn Gly Lys Gln Phe Cys Ser Thr Leu Thr Leu Asn Thr	
	85	90 95
5	Ala Gln Ala Asn His Thr Gly Phe Tyr Ser Cys Lys Tyr Leu Ala Val	
	100	105 110
	Pro Thr Ser Lys Lys Lys Glu Thr Glu Ser Ala Ile Tyr Ile Phe Ile	
10	115	120 125
	Ser Asp Thr Gly Arg Pro Phe Val Glu Met Tyr Ser Glu Ile Pro Glu	
	130	135 140
15	Ile Ile His Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val	
	145	150 155 160
	Thr Ser Pro Asn Ile Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr	
	165	170 175
20	Leu Ile Pro Asp Gly Lys Arg Ile Ile Trp Asp Ser Arg Lys Gly Phe	
	180	185 190
	Ile Ile Ser Asn Ala Thr Tyr Lys Glu Ile Gly Leu Leu Thr Cys Glu	
25	195	200 205
	Ala Thr Val Asn Gly His Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg	
	210	215 220
30	Gln Thr Asn Thr Ile Ile Asp Val Gln Ile Ser Thr Pro Arg Pro Val	
	225	230 235 240
	Lys Leu Leu Arg Gly His Thr Leu Val Leu Asn Cys Thr Ala Thr Thr	
	245	250 255

- 50 -

	Pro Leu Asn Thr Arg Val Gln Met Thr Trp Ser Tyr Pro Asp Glu Lys
	260 265 270
5	Asn Lys Arg Ala Ser Val Arg Arg Arg Ile Asp Gln Ser Asn Ser His
	275 280 285
	Ala Asn Ile Phe Tyr Ser Val Leu Thr Ile Asp Lys Met Gln Asn Lys
10	290 295 300
	Asp Lys Gly Leu Tyr Thr Cys Arg Val Arg Ser Gly Pro Ser Phe Lys
	305 310 315 320
	Ser Val Asn Thr Ser Val His Ile Tyr Asp Lys Ala Phe Ile Thr Val
15	325 330 335
	Lys His Arg Lys Gln Gln Val Leu Glu Thr Val Ala Gly Lys Arg Ser
	340 345 350
20	Tyr Arg Leu Ser Met Lys Val Lys Ala Phe Pro Ser Pro Glu Val Val
	355 360 365
	Trp Leu Lys Asp Gly Leu Pro Ala Thr Glu Lys Ser Ala Arg Tyr Leu
25	370 375 380
	Thr Arg Gly Tyr Ser Leu Ile Ile Lys Asp Val Thr Glu Glu Asp Ala
	385 390 395 400
	Gly Asn Tyr Thr Ile Leu Leu Ser Ile Lys Gln Ser Asn Val Phe Lys
30	405 410 415
	Asn Leu Thr Ala Thr Leu Ile Val Asn Val Lys Pro Gln Ile Tyr Glu
	420 425 430

- 51 -

	Lys Ala Val Ser Ser Phe Pro Asp Pro Ala Leu Tyr Pro Leu Gly Ser
	435 440 445
5	Arg Gln Ile Leu Thr Cys Thr Ala Tyr Gly Ile Pro Gln Pro Thr Ile
	450 455 460
	Lys Trp Phe Trp His Pro Cys Asn His Asn His Ser Glu Ala Arg Cys
10	465 470 475 480
	Asp Phe Cys Ser Asn Asn Glu Glu Ser Phe Ile Leu Asp Ala Asp Ser
	485 490 495
15	Asn Met Gly Asn Arg Ile Glu Ser Ile Thr Gln Arg Met Ala Ile Ile
	500 505 510
	Glu Gly Lys Asn Lys Met Ala Ser Thr Leu Val Val Ala Asp Ser Arg
	515 520 525
20	Ile Ser Gly Ile Tyr Ile Cys Ile Ala Ser Asn Lys Val Gly Thr Val
	530 535 540
	Gly Arg Asn Ile Ser Phe Tyr Ile Thr Asp Val Pro Asn Gly Phe His
25	545 550 555 560
	Val Asn Leu Glu Lys Met Pro Thr Glu Gly Glu Asp Leu Lys Leu Ser
	565 570 575
30	Cys Thr Val Asn Lys Phe Leu Tyr Arg Asp Val Thr Trp Ile Leu Leu
	580 585 590
	Arg Thr Val Asn Asn Arg Thr Met His Tyr Ser Ile Ser Lys Gln Lys
	595 600 605

- 52 -

Met Ala Ile Thr Lys Glu His Ser Ile Thr Leu Asn Leu Thr Ile Met  
610 615 620

5 Asn Val Ser Leu Gln Asp Ser Gly Thr Tyr Ala Cys Arg Ala Arg Asn  
625 630 635 640

Val Tyr Thr Gly Glu Glu Ile Leu Gln Lys Lys Glu Ile Thr Ile Arg  
645 650 655

10 Gly Glu His Cys Asn Lys Lys Ala Val Phe Ser Arg Ile Ser Lys Phe  
660 665 670

Lys Ser Thr Arg Asn Asp Cys Thr Thr Gln Ser Asn Val Lys His  
15 675 680 685

## (2) INFORMATION FOR SEQ ID NO:7:

- (1) SEQUENCE CHARACTERISTICS:
- 20 (A) LENGTH: 36 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear
- 25 (11) MOLECULE TYPE: DNA (genomic)

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:7:

30

TTTTGGATCC CTGCAGACAG ATCTACGTTT GAGAAC

- 53 -

## (2) INFORMATION FOR SEQ ID NO:8:

## (i) SEQUENCE CHARACTERISTICS:

5

(A) LENGTH: 32 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

10

(ii) MOLECULE TYPE: DNA (genomic)

## (x1) SEQUENCE DESCRIPTION: SEQ ID NO:8:

15

TTTGGATCC TTAACGCTCT AGGACTGTGA GC

32

## (2) INFORMATION FOR SEQ ID NO:9:

20

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 31 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

25

(ii) MOLECULE TYPE: DNA (genomic)

30

## (x1) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TTTGGATCC AACGGTCCCT AGGATGATGA C

31

- 54 -

## (2) INFORMATION FOR SEQ ID NO:10:

## (i) SEQUENCE CHARACTERISTICS:

5

(A) LENGTH: 23 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

10

## (ii) MOLECULE TYPE: DNA (genomic)

## (x1) SEQUENCE DESCRIPTION: SEQ ID NO:10:

15

AGCACCTTGG TTGTGGCTGA CTC

23

## (2) INFORMATION FOR SEQ ID NO:11:

## (i) SEQUENCE CHARACTERISTICS:

20

(A) LENGTH: 32 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

25

## (ii) MOLECULE TYPE: DNA (genomic)

## (x1) SEQUENCE DESCRIPTION: SEQ ID NO:11:

30

TTTGGATCC TTAGATAAGG AGGGTTAATA GG

32



- 55 -

## (2) INFORMATION FOR SEQ ID NO:12:

## (i) SEQUENCE CHARACTERISTICS:

5

(A) LENGTH: 661 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

10

(ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

15

Ser Lys Leu Lys Asp Pro Glu Leu Ser Leu Lys Gly Thr Gln His Ile

1

5

10

15

Met Gln Ala Gly Gln Thr Leu His Leu Gln Cys Arg Gly Glu Ala Ala

20

20

25

30

His Lys Trp Ser Leu Pro Glu Met Val Ser Lys Glu Ser Glu Arg Leu

35

40

45

25

Ser Ile Thr Lys Ser Ala Cys Gly Arg Asn Gly Lys Gln Phe Cys Ser

50

55

60

Thr Leu Thr Leu Asn Thr Ala Gln Ala Asn His Thr Gly Phe Tyr Ser

65

70

75

80

30

Cys Lys Tyr Leu Ala Val Pro Thr Ser Lys Lys Lys Glu Thr Glu Ser

85

90

95

- 56 -

	Ala Ile Tyr Ile Phe Ile Ser Asp Thr Gly Arg Pro Phe Val Glu Met	
	100	105 110
5	Tyr Ser Glu Ile Pro Glu Ile Ile His Met Thr Glu Gly Arg Glu Leu	
	115	120 125
	Val Ile Pro Cys Arg Val Thr Ser Pro Asn Ile Thr Val Thr Leu Lys	
	130	135 140
10	Lys Phe Pro Leu Asp Thr Leu Ile Pro Asp Gly Lys Arg Ile Ile Trp	
	145	150 155 160
	Asp Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr Tyr Lys Glu Ile	
15	165	170 175
	Gly Leu Leu Thr Cys Glu Ala Thr Val Asn Gly His Leu Tyr Lys Thr	
	180	185 190
20	Asn Tyr Leu Thr His Arg Gln Thr Asn Thr Ile Ile Asp Val Gln Ile	
	195	200 205
	Ser Thr Pro Arg Pro Val Lys Leu Leu Arg Gly His Thr Leu Val Leu	
	210	215 220
25	Asn Cys Thr Ala Thr Thr Pro Leu Asn Thr Arg Val Gln Met Thr Trp	
	225	230 235 240
	Ser Tyr Pro Asp Glu Lys Asn Lys Arg Ala Ser Val Arg Arg Arg Ile	
30	245	250 255
	Asp Gln Ser Asn Ser His Ala Asn Ile Phe Tyr Ser Val Leu Thr Ile	
	260	265 270

- 57 -

	Asp Lys Met Gln Asn Lys Asp Lys Gly Leu Tyr Thr Cys Arg Val Arg	
	275	280 285
5	Ser Gly Pro Ser Phe Lys Ser Val Asn Thr Ser Val His Ile Tyr Asp	
	290	295 300
	Lys Ala Phe Ile Thr Val Lys His Arg Lys Gln Gln Val Leu Glu Thr	
10	305	310 315 320
	Val Ala Gly Lys Arg Ser Tyr Arg Leu Ser Met Lys Val Lys Ala Phe	
	325	330 335
15	Pro Ser Pro Glu Val Val Trp Leu Lys Asp Gly Leu Pro Ala Thr Glu	
	340	345 350
	Lys Ser Ala Arg Tyr Leu Thr Arg Gly Tyr Ser Leu Ile Ile Lys Asp	
	355	360 365
20	Val Thr Glu Glu Asp Ala Gly Asn Tyr Thr Ile Leu Leu Ser Ile Lys	
	370	375 380
	Gln Ser Asn Val Phe Lys Asn Leu Thr Ala Thr Leu Ile Val Asn Val	
25	385	390 395 400
	Lys Pro Gln Ile Tyr Glu Lys Ala Val Ser Ser Phe Pro Asp Pro Ala	
	405	410 415
30	Leu Tyr Pro Leu Gly Ser Arg Gln Ile Leu Thr Cys Thr Ala Tyr Gly	
	420	425 430
	Ile Pro Gln Pro Thr Ile Lys Trp Phe Trp His Pro Cys Asn His Asn	
	435	440 445

- 58 -

	His Ser Glu Ala Arg Cys Asp Phe Cys Ser Asn Asn Glu Glu Ser Phe
	450                      455                      460
5	Ile Leu Asp Ala Asp Ser Asn Met Gly Asn Arg Ile Glu Ser Ile Thr
	465                      470                      475                      480
	( Gln Arg Met Ala Ile Ile Glu Gly Lys Asn Lys Met Ala Ser Thr Leu
	485                      490                      495
10	Val Val Ala Asp Ser Arg Ile Ser Gly Ile Tyr Ile Cys Ile Ala Ser
	500                      505                      510
	Asn Lys Val Gly Thr Val Gly Arg Asn Ile Ser Phe Tyr Ile Thr Asp
15	515                      520                      525
	Val Pro Asn Gly Phe His Val Asn Leu Glu Lys Met Pro Thr Glu Gly
	530                      535                      540
20	Glu Asp Leu Lys Leu Ser Cys Thr Val Asn Lys Phe Leu Tyr Arg Asp
	545                      550                      555                      560
	Val Thr Trp Ile Leu Leu Arg Thr Val Asn Asn Arg Thr Met His Tyr
	565                      570                      575
25	Ser Ile Ser Lys Gln Lys Met Ala Ile Thr Lys Glu His Ser Ile Thr
	580                      585                      590
	Leu Asn Leu Thr Ile Met Asn Val Ser Leu Gln Asp Ser Gly Thr Tyr
30	595                      600                      605
	Ala Cys Arg Ala Arg Asn Val Tyr Thr Gly Glu Glu Ile Leu Gln Lys
	610                      615                      620

- 59 -

Lys Glu Ile Thr Ile Arg Gly Glu His Cys Asn Lys Lys Ala Val Phe  
 625                      630                      635                      640

5

Ser Arg Ile Ser Lys Phe Lys Ser Thr Arg Asn Asp Cys Thr Thr Gln  
                                 645                      650                      655

Ser Asn Val Lys His  
                                 660

10

## (2) INFORMATION FOR SEQ ID NO:13:

## (i) SEQUENCE CHARACTERISTICS:

15

- (A) LENGTH: 668 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

20

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

25

Ser Glu Gln Asn Met Gln Ser Lys Val Leu Leu Ala Val Ala Leu Trp  
 1                      5                      10                      15

Leu Cys Val Glu Thr Arg Ala Ala Ser Val Gly Leu Pro Ser Val Ser  
                                 20                      25                      30

30

Leu Asp Leu Pro Arg Leu Ser Ile Gln Lys Asp Ile Leu Thr Ile Lys  
                                 35                      40                      45

- 60 -

Ala Asn Thr Thr Leu Gln Ile Thr Cys Arg Gly Gln Arg Asp Leu Asp  
50 55 60

5 Trp Leu Trp Pro Asn Asn Gln Ser Gly Ser Glu Gln Arg Val Glu Val  
65 70 75 80

Thr Glu Cys Ser Asp Gly Leu Phe Cys Lys Thr Leu Thr Ile Pro Lys  
85 90 95

10 Val Ile Gly Asn Asp Thr Gly Ala Tyr Lys Cys Phe Tyr Arg Glu Thr  
100 105 110

Asp Leu Ala Ser Val Ile Tyr Val Tyr Val Gln Asp Tyr Arg Ser Pro  
115 120 125

15 Phe Ile Ala Ser Val Ser Asp Gln His Gly Val Val Tyr Ile Thr Glu  
130 135 140

20 Asn Lys Asn Lys Thr Val Val Ile Pro Cys Leu Gly Ser Ile Ser Asn  
145 150 155 160

Leu Asn Val Ser Leu Cys Ala Arg Tyr Pro Glu Lys Arg Phe Val Pro  
165 170 175

25 Asp Gly Asn Arg Ile Ser Trp Asp Ser Lys Lys Gly Phe Thr Ile Pro  
180 185 190

Ser Tyr Met Ile Ser Tyr Ala Gly Met Val Phe Cys Glu Ala Lys Ile  
195 200 205

30 Asn Asp Glu Ser Tyr Gln Ser Ile Met Tyr Ile Val Val Val Val Gly  
210 215 220

- 61 -

	Tyr Arg Ile Tyr Asp Val Val Leu Ser Pro Ser His Gly Ile Glu Leu	
	225	230 235 240
5	Ser Val Gly Glu Lys Leu Val Leu Asn Cys Thr Ala Arg Thr Glu Leu	
	245	250 255
	Asn Val Gly Ile Asp Phe Asn Trp Glu Tyr Pro Ser Ser Lys His Gln	
	260	265 270
10	His Lys Lys Leu Val Asn Arg Asp Leu Lys Thr Gln Ser Gly Ser Glu	
	275	280 285
	Met Lys Lys Phe Leu Ser Thr Leu Thr Ile Asp Gly Val Thr Arg Ser	
15	290	295 300
	Asp Gln Gly Leu Tyr Thr Cys Ala Ala Ser Ser Gly Leu Met Thr Lys	
	305	310 315 320
20	Lys Asn Ser Thr Phe Val Arg Val His Glu Lys Pro Phe Val Ala Phe	
	325	330 335
	Gly Ser Gly Met Glu Ser Leu Val Glu Ala Thr Val Gly Glu Arg Val	
	340	345 350
25	Arg Ile Pro Ala Lys Tyr Leu Gly Tyr Pro Pro Pro Glu Ile Lys Trp	
	355	360 365
	Tyr Lys Asn Gly Ile Pro Leu Glu Ser Asn His Thr Ile Lys Ala Gly	
30	370	375 380
	His Val Leu Thr Ile Met Glu Val Ser Glu Arg Asp Thr Gly Asn Tyr	
	385	390 395 400

- 62 -

	Thr Val Ile Leu Thr Asn Pro Ile Ser Lys Glu Lys Gln Ser His Val	
	405	415
5	Val Ser Leu Val Val Tyr Val Pro Pro Gln Ile Gly Glu Lys Ser Leu	
	420	430
	Ile Ser Pro Val Asp Ser Tyr Gln Tyr Gly Thr Thr Gln Thr Leu Thr	
10	435	445
	Cys Thr Val Tyr Ala Ile Pro Pro Pro His His Ile His Trp Tyr Trp	
	450	460
15	Gln Leu Glu Glu Glu Cys Ala Asn Glu Pro Ser Gln Ala Val Ser Val	
	465	480
	Thr Asn Pro Tyr Pro Cys Glu Glu Trp Arg Ser Val Glu Asp Phe Gln	
	485	495
20	Gly Gly Asn Lys Ile Ala Val Asn Lys Asn Gln Phe Ala Leu Ile Glu	
	500	510
	Gly Lys Asn Lys Thr Val Ser Thr Leu Val Ile Gln Ala Ala Asn Val	
25	515	525
	Ser Ala Leu Tyr Lys Cys Glu Ala Val Asn Lys Val Gly Arg Gly Glu	
	530	540
30	Arg Val Ile Ser Phe His Val Thr Arg Gly Pro Glu Ile Thr Leu Gln	
	545	560
	Pro Asp Met Gln Pro Thr Glu Gln Glu Ser Val Ser Leu Trp Cys Thr	
	565	575



- 63 -

Ala Asp Arg Ser Thr Phe Glu Asn Leu Thr Trp Tyr Lys Leu Gly Pro  
580 585 590

5 Gln Pro Leu Pro Ile His Val Gly Glu Leu Pro Thr Pro Val Cys Lys  
595 600 605

Asn Leu Asp Thr Leu Trp Lys Leu Asn Ala Thr Met Phe Ser Asn Ser  
610 615 620

10 Thr Asn Asp Ile Leu Ile Met Glu Leu Lys Asn Ala Ser Leu Gln Asp  
625 630 635 640

Gln Gly Asp Tyr Val Cys Leu Ala Gln Asp Arg Lys Thr Lys Lys Arg  
15 645 650 655

His Cys Val Val Arg Gln Leu Thr Val Leu Glu Arg  
660 665

20 (2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 780 amino acids  
(B) TYPE: amino acid  
25 (C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

30

- 64 -

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

5	Met Val Ser Tyr Trp Asp Thr Gly Val Leu Leu Cys Ala Leu Leu Ser
	1                      5                      10                      15
	Cys Leu Leu Leu Thr Gly Ser Ser Ser Gly Ser Lys Leu Lys Asp Pro
	20                      25                      30
10	Glu Leu Ser Leu Lys Gly Thr Gln His Ile Met Gln Ala Gly Gln Thr
	35                      40                      45
	Leu His Leu Gln Cys Arg Gly Glu Ala Ala His Lys Trp Ser Leu Pro
15	50                      55                      60
	Glu Met Val Ser Lys Glu Ser Glu Arg Leu Ser Ile Thr Lys Ser Ala
	65                      70                      75                      80
20	Cys Gly Arg Asn Gly Lys Gln Phe Cys Ser Thr Leu Thr Leu Asn Thr
	85                      90                      95
	Ala Gln Ala Asn His Thr Gly Phe Tyr Ser Cys Lys Tyr Leu Ala Val
	100                      105                      110
25	Pro Thr Ser Lys Lys Lys Glu Thr Glu Ser Ala Ile Tyr Ile Phe Ile
	115                      120                      125
	Ser Asp Thr Gly Arg Pro Phe Val Glu Met Tyr Ser Glu Ile Pro Glu
30	130                      135                      140
	Ile Ile His Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val
	145                      150                      155                      160

- 65 -

	Thr Ser Pro Asn Ile Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr		
	165	170	175
5	Leu Ile Pro Asp Gly Lys Arg Ile Ile Trp Asp Ser Arg Lys Gly Phe		
	180	185	190
	Ile Ile Ser Asn Ala Thr Tyr Lys Glu Ile Gly Leu Leu Thr Cys Glu		
10	195	200	205
	Ala Thr Val Asn Gly His Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg		
	210	215	220
15	Gln Thr Asn Thr Ile Ile Asp Val Gln Ile Ser Thr Pro Arg Pro Val		
	225	230	235 240
	Lys Leu Leu Arg Gly His Thr Leu Val Leu Asn Cys Thr Ala Thr Thr		
	245	250	255
20	Pro Leu Asn Thr Arg Val Gln Met Thr Trp Ser Tyr Pro Asp Glu Lys		
	260	265	270
	Asn Lys Arg Ala Ser Val Arg Arg Arg Ile Asp Gln Ser Asn Ser His		
25	275	280	285
	Ala Asn Ile Phe Tyr Ser Val Leu Thr Ile Asp Lys Met Gln Asn Lys		
	290	295	300
30	Asp Lys Gly Leu Tyr Thr Cys Arg Val Arg Ser Gly Pro Ser Phe Lys		
	305	310	315 320
	Ser Val Asn Thr Ser Val His Ile Tyr Asp Lys Ala Phe Ile Thr Val		
	325	330	335

- 66 -

Lys His Arg Lys Gln Gln Val Leu Glu Thr Val Ala Gly Lys Arg Ser  
 340 345 350

5 Tyr Arg Leu Ser Met Lys Val Lys Ala Phe Pro Ser Pro Glu Val Val  
 355 360 365

Trp Leu Lys Asp Gly Leu Pro Ala Thr Glu Lys Ser Ala Arg Tyr Leu  
 10 370 375 380

Thr Arg Gly Tyr Ser Leu Ile Ile Lys Asp Val Thr Glu Glu Asp Ala  
 385 390 395 400

15 Gly Asn Tyr Thr Ile Leu Leu Ser Ile Lys Gln Ser Asn Val Phe Lys  
 405 410 415

Asn Leu Thr Ala Thr Leu Ile Val Asn Val Lys Pro Gln Ile Tyr Glu  
 420 425 430

20 Lys Ala Val Ser Ser Phe Pro Asp Pro Ala Leu Tyr Pro Leu Gly Ser  
 435 440 445

Arg Gln Ile Leu Thr Cys Thr Ala Tyr Gly Ile Pro Gln Pro Thr Ile  
 25 450 455 460

Lys Trp Phe Trp His Pro Cys Asn His Asn His Ser Glu Ala Arg Cys  
 465 470 475 480

30 Asp Phe Cys Ser Asn Asn Glu Glu Ser Phe Ile Leu Asp Ala Asp Ser  
 485 490 495

Asn Met Gly Asn Arg Ile Glu Ser Ile Thr Gln Arg Met Ala Ile Ile  
 500 505 510

- 67 -

	Glu Gly Lys Asn Lys Met Ala Ser Thr Leu Val Val Ala Asp Ser Arg
	515 520 525
5	Ile Ser Gly Ile Tyr Ile Cys Ile Ala Ser Asn Lys Val Gly Thr Val
	530 535 540
	Gly Arg Asn Ile Ser Phe Tyr Ile Thr Asp Val Pro Asn Gly Phe His
10	545 550 555 560
	Val Asn Leu Glu Lys Met Pro Thr Glu Gly Glu Asp Leu Lys Leu Ser
	565 570 575
15	Cys Thr Val Asn Lys Phe Leu Tyr Arg Asp Val Thr Trp Ile Leu Leu
	580 585 590
	Arg Thr Val Asn Asn Arg Thr Met His Tyr Ser Ile Ser Lys Gln Lys
	595 600 605
20	Met Ala Ile Thr Lys Glu His Ser Ile Thr Leu Asn Leu Thr Ile Met
	610 615 620
	Asn Val Ser Leu Gln Asp Ser Gly Thr Tyr Ala Cys Arg Ala Arg Asn
25	625 630 635 640
	Val Tyr Thr Gly Glu Glu Ile Leu Gln Lys Lys Glu Ile Thr Ile Arg
	645 650 655
30	Asp Gln Glu Ala Pro Tyr Leu Leu Arg Asn Leu Ser Asp His Thr Val
	660 665 670
	Ala Ile Ser Ser Ser Thr Thr Leu Asp Cys His Ala Asn Gly Val Pro
	675 680 685

- 68 -

Glu Pro Gln Ile Thr Trp Phe Lys Asn Asn His Lys Ile Gln Gln Glu  
 690 695 700

5 Pro Gly Ile Ile Leu Gly Pro Gly Ser Ser Thr Leu Phe Ile Glu Arg  
 705 710 715 720

Val Thr Glu Glu Asp Glu Gly Val Tyr His Cys Lys Ala Thr Asn Gln  
 725 730 735

10 Lys Gly Ser Val Glu Ser Ser Ala Tyr Leu Thr Val Gln Gly Thr Ser  
 740 745 750

Asp Lys Ser Asn Leu Glu Leu Ile Thr Leu Thr Cys Thr Cys Val Ala  
 15 755 760 765

Ala Thr Leu Phe Trp Leu Leu Leu Thr Leu Leu Ile  
 770 775 780

20 (2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- 25 (A) LENGTH: 788 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

30

- 69 -

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:15:

5	Met Gln Ser Lys Val Leu Leu Ala Val Ala Leu Trp Leu Cys Val Glu
	1                      5                      10                      15
	Thr Arg Ala Ala Ser Val Gly Leu Pro Ser Val Ser Leu Asp Leu Pro
	20                      25                      30
10	Arg Leu Ser Ile Gln Lys Asp Ile Leu Thr Ile Lys Ala Asn Thr Thr
	35                      40                      45
	Leu Gln Ile Thr Cys Arg Gly Gln Arg Asp Leu Asp Trp Leu Trp Pro
15	50                      55                      60
	Asn Asn Gln Ser Gly Ser Glu Gln Arg Val Glu Val Thr Glu Cys Ser
	65                      70                      75                      80
20	Asp Gly Leu Phe Cys Lys Thr Leu Thr Ile Pro Lys Val Ile Gly Asn
	85                      90                      95
	Asp Thr Gly Ala Tyr Lys Cys Phe Tyr Arg Glu Thr Asp Leu Ala Ser
	100                      105                      110
25	Val Ile Tyr Val Tyr Val Gln Asp Tyr Arg Ser Pro Phe Ile Ala Ser
	115                      120                      125
	Val Ser Asp Gln His Gly Val Val Tyr Ile Thr Glu Asn Lys Asn Lys
30	130                      135                      140
	Thr Val Val Ile Pro Cys Leu Gly Ser Ile Ser Asn Leu Asn Val Ser
	145                      150                      155                      160

- 70 -

	Leu Cys Ala Arg Tyr Pro Glu Lys Arg Phe Val Pro Asp Gly Asn Arg
	165 170 175
5	Ile Ser Trp Asp Ser Lys Lys Gly Phe Thr Ile Pro Ser Tyr Met Ile
	180 185 190
	Ser Tyr Ala Gly Met Val Phe Cys Glu Ala Lys Ile Asn Asp Glu Ser
	195 200 205
10	Tyr Gln Ser Ile Met Tyr Ile Val Val Val Val Gly Tyr Arg Ile Tyr
	210 215 220
	Asp Val Val Leu Ser Pro Ser His Gly Ile Glu Leu Ser Val Gly Glu
15	225 230 235 240
	Lys Leu Val Leu Asn Cys Thr Ala Arg Thr Glu Leu Asn Val Gly Ile
	245 250 255
20	Asp Phe Asn Trp Glu Tyr Pro Ser Ser Lys His Gln His Lys Lys Leu
	260 265 270
	Val Asn Arg Asp Leu Lys Thr Gln Ser Gly Ser Glu Met Lys Lys Phe
	275 280 285
25	Leu Ser Thr Leu Thr Ile Asp Gly Val Thr Arg Ser Asp Gln Gly Leu
	290 295 300
	Tyr Thr Cys Ala Ala Ser Ser Gly Leu Met Thr Lys Lys Asn Ser Thr
30	305 310 315 320
	Phe Val Arg Val His Glu Lys Pro Phe Val Ala Phe Gly Ser Gly Met
	325 330 335



- 71 -

	Glu Ser Leu Val Glu Ala Thr Val Gly Glu Arg Val Arg Ile Pro Ala	
	340	345 350
5	Lys Tyr Leu Gly Tyr Pro Pro Pro Glu Ile Lys Trp Tyr Lys Asn Gly	
	355	360 365
	Ile Pro Leu Glu Ser Asn His Thr Ile Lys Ala Gly His Val Leu Thr	
10	370	375 380
	Ile Met Glu Val Ser Glu Arg Asp Thr Gly Asn Tyr Thr Val Ile Leu	
	385	390 395 400
15	Thr Asn Pro Ile Ser Lys Glu Lys Gln Ser His Val Val Ser Leu Val	
	405	410 415
	Val Tyr Val Pro Pro Gln Ile Gly Glu Lys Ser Leu Ile Ser Pro Val	
	420	425 430
20	Asp Ser Tyr Gln Tyr Gly Thr Thr Gln Thr Leu Thr Cys Thr Val Tyr	
	435	440 445
	Ala Ile Pro Pro Pro His His Ile His Trp Tyr Trp Gln Leu Glu Glu	
25	450	455 460
	Glu Cys Ala Asn Glu Pro Ser Gln Ala Val Ser Val Thr Asn Pro Tyr	
	465	470 475 480
30	Pro Cys Glu Glu Trp Arg Ser Val Glu Asp Phe Gln Gly Gly Asn Lys	
	485	490 495
	Ile Ala Val Asn Lys Asn Gln Phe Ala Leu Ile Glu Gly Lys Asn Lys	
	500	505 510

- 72 -

	Thr Val Ser Thr Leu Val Ile Gln Ala Ala Asn Val Ser Ala Leu Tyr	
	515	520 525
5	Lys Cys Glu Ala Val Asn Lys Val Gly Arg Gly Glu Arg Val Ile Ser	
	530	535 540
	Phe His Val Thr Arg Gly Pro Glu Ile Thr Leu Gln Pro Asp Met Gln	
	545	550 555 560
10	Pro Thr Glu Gln Glu Ser Val Ser Leu Trp Cys Thr Ala Asp Arg Ser	
	565	570 575
	Thr Phe Glu Asn Leu Thr Trp Tyr Lys Leu Gly Pro Gln Pro Leu Pro	
15	580	585 590
	Ile His Val Gly Glu Leu Pro Thr Pro Val Cys Lys Asn Leu Asp Thr	
	595	600 605
20	Leu Trp Lys Leu Asn Ala Thr Met Phe Ser Asn Ser Thr Asn Asp Ile	
	610	615 620
	Leu Ile Met Glu Leu Lys Asn Ala Ser Leu Gln Asp Gln Gly Asp Tyr	
25	625	630 635 640
	Val Cys Leu Ala Gln Asp Arg Lys Thr Lys Lys Arg His Cys Val Val	
	645	650 655
	Arg Gln Leu Thr Val Leu Glu Arg Val Ala Pro Thr Ile Thr Gly Asn	
30	660	665 670
	Leu Glu Asn Gln Thr Thr Ser Ile Gly Glu Ser Ile Glu Val Ser Cys	
	675	680 685

- 73 -

	Thr Ala Ser Gly Asn Pro Pro Pro Gln Ile Met Trp Phe Lys Asp Asn
	690                      695                      700
5	Glu Thr Leu Val Glu Asp Ser Gly Ile Val Leu Lys Asp Gly Asn Arg
	705                      710                      715                      720
	Asn Leu Thr Ile Arg Arg Val Arg Lys Glu Asp Glu Gly Leu Tyr Cys
	725                      730                      735
10	Gln Ala Cys Ser Val Leu Gly Cys Ala Lys Val Glu Ala Phe Phe Ile
	740                      745                      750
	Ile Glu Gly Ala Gln Glu Lys Thr Asn Leu Glu Ile Ile Ile Leu Val
15	755                      760                      765
	Gly Thr Thr Val Ile Ala Met Phe Phe Trp Leu Leu Leu Val Ile Ile
	770                      775                      780
20	Leu Gly Thr Val
	785

## (2) INFORMATION FOR SEQ ID NO:16:

- 25 (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 2264 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

30

(ii) MOLECULE TYPE: DNA (genomic)

- 74 -

## (x1) SEQUENCE DESCRIPTION: SEQ ID NO:16:

5	GGTGTGGTCG CTGCGTTTCC TCTGCCTGCG CCGGGCATCA CTTGCGCGCC GCAGAAAGTC	60
	CGTCTGGCAG CCTGGATATC CTCTCCTACC GGCACCCGCA GACGCCCTG CAGCCGCGGT	120
	CGGCGCCCGG GCTCCCTAGC CCTGTGCGCT CAACTGTCTT GCCTGCGGG GTGCCGCGAG	180
10	TTCCACCTCC GCGCCTCCTT CTCTAGACAG GCGCTGGGAG AAAGAACCGG CTCCCGAGTT	240
	CCGGCATTTC GCCCGGCTCG AGGTGCAGGA TGCAGAGCAA GGTGCTGCTG GCCGTCGCCC	300
15	TGTGGCTCTG CGTGGAGACC CGGGCCGCCT CTGTGGGTTT GCCTAGTGTT TCTCTTGATC	360
	TGCCCAGGCT CAGCATACAA AAAGACATAC TTACAATTAA GGCTAATACA ACTCTTCAAA	420
	TTACTTGCAg GGGACAGAGG GACTTGGACT GGCTTTGGCC CAATAATCAG AGTGGCAGTG	480
20	AGCAAAGGCT GGAGGTGACT GAGTGCAGCG ATGGCCTCTT CTGTAAGACA CTCACAATTC	540
	CAAAAGTGAT CGGAAATGAC ACTGGAGCCT ACAAGTGCTT CTACCGGGAA ACTGACTTGG	600
25	CCTCGGTCAT TTATGTCTAT GTTCAAGATT ACAGATCTCC ATTTATTGCT TCTGTTAGTG	660
	ACCAACATGG AGTCGTGTAC ATTACTGAGA AAAAAACAA AACTGTGGTG ATTCCATGTC	720
	TCGGGTCCAT TTCAAATCTC AACGTGTAC TTTGTGCAAG ATACCCAGAA AAGAGATTG	780
30	TTCTGTATGG TAACAGAATT TCCTGGGACA GCAAGAAGGG CTTTACTATT CCCAGCTACA	840
	TGATCAGCTA TGCTGGCATG GTCTTCTGTG AAGCAAAAAT TAATGATGAA AGTTACCACT	900

- 75 -

CTATTATGTA CATAGTTGTC GTTGTAGGGT ATAGGATTTA TGATGTGGTT CTGAGTCCGT 960

5 CTATGGAAT TGAACATCT GTTGGAGAAA AGCTTGCTT AAATTGTACA GCAAGAACTG 1020

AACTAAATGT GGGGATTGAC TTCAACTGGG AATACCCTTC TTCGAAGCAT CAGCATAAGA 1080

AACTGTAAA CCGAGACCTA AAAACCCAGT CTGGGAGTGA GATGAAGAAA TTTTGTAGCA 1140

10 CCTTAACTAT AGATGGTGTG ACCCGGAGTG ACCAAGGATT GTACACCTGT GCAGCATCCA 1200

GTGGGCTGAT GACCAAGAAG AACAGCACAT TTGTCAGGGT CCATGAAAAA CCTTTTGTG 1260

15 CTTTGTGAAG TGGCATGGAA TCTCTGGTG AAGCCACGGT GGGGAGCGT GTCAGATCC 1320

CTGCGAAGTA CCTTGGTTAC CCACCCAG AAATAAATG GTATAAAT GGAATACCC 1380

TTGAGTCAA TCACACAATT AAAGCGGGC ATGTACTGAC GATTATGGAA GTGAGTAAA 1440

20 GAGACACAGG AAATTACACT GTCATCTTA CCAATCCAT TTCAAAGGAG AAGCAGAGCC 1500

ATGTGGTCTC TCTGGTTGTG TATGTCCAC CCCAGATTG TGAGAAATCT CTAATCTCTC 1560

25 CTGTGGATTC CTACCAGTAC GGCACCACTC AAACGCTGAC ATGTACGGTC TATGCCATTC 1620

CTCCCCGCA TCACATCCAC TGGTATTGGC AGTTGGAGGA AGAGTGGCC AACGAGCCCA 1680

GCCAAGCTGT CTCAGTGACA AACCCATACC CTTGTGAAGA ATGGAGAAST GTGGAGGACT 1740

30 TCCAGGGAGG AAATAAAT GCGTTAATA AAAATCAATT TGCTCTAATT GAAGGAAAAA 1800

ACAAAACTGT AAGTACCCTT GTTATCCAAG CGGCAATGT GTCAGCTTG TACAAATGTG 1860

AAGCGGTCAA CAAAGTCGGG AGAGGAGAGA GGGTGATCTC CTTCCACGTG ACCAGGGGTC 1920

- 76 -

5 CTGAAATTAC TTTGCAACCT GACATGCAGC CCACTGAGCA GGAGAGCGTG TCTTTGTGGT 1980  
GCACTGCAGA CAGATCTACG TTTGAGAACC TCACATGGTA CAAGCTTGGC CCACAGCCTC 2040  
TGCCAATCCA TGTGGGAGAG TTGCCCACAC CTGTTTGCAA GAACTTGGAT ACTCTTTGGA 2100  
AATTGAATGC CACCATGTTT TCTAATAGCA CAAATGACAT TTTGATCATG GAGCTTAAGA 2160  
10 ATGCATCCTT GCAGGACCAA GGAGACTATG TCTGCCTTGC TCAAGACAGG AAGACCAAGA 2220  
AAAGACATTG CGTGGTCAGG CAGCTCACAG TCCTAGAGCG TTAA 2264

## (2) INFORMATION FOR SEQ ID NO:17:

15

## (1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2352 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

20

(D) TOPOLOGY: linear

(11) MOLECULE TYPE: DNA (genomic)

25

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

60 GCGTCACCA TGGTCAGCTA CTGGGACACC GGGGTCCTGC TGTGCGCGCT GCTCAGCTGT  
30 CTGCTTCTCA CAGGATCTAG TTCAGGTTC AATTAAAG ATCCTGAACT GAGTTTAAAA 120  
GGCAGCCAGC ACATCATGCA AGCAGGCCAG AACTGCATC TCCAATGCAG GGGGGAAGCA 180  
GCCATAAAT GGTCTTTGCC TGAAATGCTG AGTAAGGAAA GCGAAGGCT GAGCATAACT 240

- 77 -

	AAATCTGCCT GTGGAAGAAA TGGCAAACAA TTCTGCAGTA CTTTAACTT GAACACAGCT	300
5	CAAGCAAACC ACACTGGCTT CTACAGCTGC AAATATCTAG CTGTACCTAC TTCAAAGAAG	360
	AAGGAAACAG AATCTGCAAT CTATATATTT ATTAGTGATA CAGGTAGACC TTTCGTAGAG	420
	ATGTACAGTG AAATCCCCGA AATTATACAC ATGACTGAAG GAAGGGAGCT CGTCATTCCC	480
10	TGCCGGGTTA CGTCACCTAA CATCACTGTT ACTTTAAAAA AGTTTCCACT TGACACTTTG	540
	ATCCCTGATG GAAAACGCAT AATCTGGGAC AGTAGAAAGG GCTTCATCAT ATCAAATGCA	600
15	ACGTACAAAG AAATAGGGCT TCTGACCTGT GAAGCAACAG TCAATGGGCA TTTGTATAAG	660
	ACAACTATC TCACACATCG ACAAACCAAT ACAATCATAG ATGTCCAAAT AAGCACACCA	720
	CGCCCACTCA AATTACTTAG AGGCCATACT CTTGTCTCA ATTGTACTGC TACCACTCCC	780
20	TTGAACACGA GAGTTCAAAT GACCTGGAGT TACCCTGATG AAAAAATAA GAGAGCTTCC	840
	GTAAGGCGAC GAATTGACCA AAGCAATTCC CATGCCAACA TATTCTACAG TGTTCTTACT	900
25	ATTGACAAAA TGCAGAACAA AGACAAAGGA CTTTATACTT GTCGTGTAAG GAGTGGACCA	960
	TCATTCAAAT CTGTTAACAC CTCAGTGCAT ATATATGATA AAGCATTCACT CACTGTGAAA	1020
	CATCGAAAAC AGCAGGTGCT TGAACCCTA GCTGGCAAGC GGTCTTACCG GCTCTCTATG	1080
30	AAAGTGAAGG CATTTCCTTC GCCGGAAGTT STATGGTTAA AAGATGGGTT ACCTGCGACT	1140
	GAGAAATCTG CTCGCTATTT GACTCGTGGC TACTCGTTAA TTATCAAGGA CGTAACTGAA	1200
	GAGGATSCAG GGAATTATAC AATCTTGCTG AGCATAAAAC AGTCAAAATGT GTTTAAAAAC	1260

- 78 -

	CTCACTGCCA CTCTAATTGT CAATGTGAAA CCCAGATT ACGAAAAGGC CGTGTCTCG	1320
5	TTTCAGACC CGGCTCTCTA CCCACTGGG AGCAGACAAA TCCTGACTTG TACCGCATAT	1380
	GGTATCCCTC AACCTACAAT CAAGTGGTC TGGCACCCCT GTAACCATAA TCATTCCGAA	1440
	GCAAGGTGTG ACTTTTGTTC CAATAATGAA GAGTCCTTGA TCCTGGATGC TGACAGCAAC	1500
10	ATGGGAAACA GAATTGAGAG CATCACTCAG CGCATGGCAA TAATAGAAGG AAAGAATAAG	1560
	ATGGCTAGCA CCTTGGTTGT GGCTGACTCT AGAATTTCTG GAATCTACAT TTGCATAGCT	1620
15	TCCAATAAG TTGGGACTGT GGAAGAAAC ATAAGCTTTT ATATCACAGA TGTGCCAAAT	1680
	GGGTTTCATG TTAACCTGGA AAAATGCCG ACGGAAGGAG AGGACCTGAA ACTGTCTTGC	1740
	ACAGTTAACA AGTCTTATA CAGAGAGTT ACTTGGATT TACTGCGGAC AGTTAATAAC	1800
20	AGAACAATGC ACTACAGTAT TAGCAAGCAA AAAATGGCCA TCACTAAGGA GCACTCCATC	1860
	ACTCTTAATC TTACCATCAT GAATGTTTCC CTGCAAGATT CAGGCACCTA TGCCTGCAGA	1920
25	GCCAGGAATG TATACACAGG GGAAGAAATC CTCCAGAAGA AAGAAATTAC AATCAGAGAT	1980
	CAGGAAGCAC CATACCTCCT GCGAAACCTC AGTGATCACA CAGTGGCCAT CAGCAGTTCC	2040
	ACCACTTTAG ACTGTCTATG TAATGGTGC CCCGAGCCTC AGATCACTTG GTTTAAAAAC	2100
30	AACCACAAAA TACAACAAGA GCCTGGAATT ATTTAGGAC CAGGAAGCAG CACGCTGTTT	2160
	ATTGAAAGAG TCACAGAAGA GGATGAAGGT GTCTATCACT GCAAAGCCAC CAACCAGAAG	2220
	GGCTCTGTGG AAAGTTCAGC ATACCTCACT GTTCAAGGAA CCTCGGACAA GTCTAATCTG	2280



- 79 -

GAGCTGATCA CTCTAACATG CACCTGTGTG GCTGC6ACTC TCTTCTGGCT CCTATTAACC 2340

CTCCTTATCT AA 2352

5

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

10

(A) LENGTH: 2383 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

20

CTCGAGGTGC AGGATGCAGA GCAAGGTGCT GCTGGCCGTC GCCCTGTGGC TCTGCGTGA 60

GACCCGGGCC GCCTCTGTGG GTTGCCTAG TGTCTCTTT GATCTGCCA GGCTCAGCAT 120

25

ACAAAAAGAC ATACTTACAA TTAAGGCTAA TACAACCTTT CAAATTACTT GCAGGGGACA 180

GAGGGACTTG GACTGGCTTT GGCCCAATAA TCAGAGTGGC AGTGAGCAAA GGGTGGAGGT 240

GACTGAGTGC AGCGATGGCC TCTTCTGTAA GACACTCACA ATTCCAAAAG TGATCGGAAA 300

30

TGACACTGGA GCCTACAAGT GCTTCTACCG GGAAACTGAC TTGGCCTCGG TCATTATGT 360

CTATGTTCAA GATTACAGAT CTCCATTAT TGCTTCTGTT AGTGACCAAC ATGGAGTCGT 420

GTACATTACT GAGAACAAA ACAAACTGT GGTGATTCCA TGTCTCGGT CCATTTCAAA 480

- 80 -

5  
10  
15  
20  
25  
30

TCTCAACGTG TCACCTTGTG CAAGATACCC AGAAAAGAGA TTTGTCTCTG ATGGTAACAG 540  
AATTCCTGG GACAGCAAGA AGGGCTTTAC TATCCCAGC TACATGATCA GCTATGCTGG 600  
CATGGTCTTC TGTGAAGCAA AAATTAATGA TGAAAGTTAC CAGTCTATTA TGTACATAGT 660  
TGTCGTTGTA GGGTATAGGA TTTATGATGT GGTCTGAGT CCGTCTCATG GAATTGAACT 720  
ATCTGTTGGA GAAAAGCTTG TCTTAAATTG TACAGCAAGA ACTGAACTAA ATGTGGGGAT 780  
TGACTTCAAC TGGGAATACC CTTCTTCGAA GCATCAGCAT AAGAACTTG TAAACCGAGA 840  
CCTAAAAACC CAGTCTGGGA GTGAGATGAA GAAATTTTGG AGCACCTTAA CTATAGATGG 900  
TGTAACCCGG AGTGACCAAG GATTGTACAC CTGTGCAGCA TCCAGTGGGC TGATGACCAA 960  
GAAGAACAGC ACATTGTCA GGGTCCATGA AAAACCTTTT GTTGCTTTTG GAAGTGGCAT 1020  
GGAATCTCTG GTGGAAGCCA CGGTGGGGGA GCGTGTGAGA ATCCCTGCCA AGTACCTTGG 1080  
TTACCCACCC CCAGAAATAA AATGATATAA AAATGGAATA CCCCTTGAGT CCAATCACAC 1140  
AATTAAAGCG GGGCATGTAC TGACGATTAT GGAAGTGAGT GAAAGAGACA CAGGAAATTA 1200  
CACTGTCATC CTTACCAATC CCATTTCAA GGAGAAGCAG AGCCATGTGG TCTCTCTGGT 1260  
TGTGTATGTC CCACCCGAGA TTGGTGAGAA ATCTCTAATC TCTCTGTGG ATTCTACCA 1320  
GTACGGCACC ACTCAAACGC TGACATGTAC GGTCTATGCC ATTCTCCCC CGCATCACAT 1380  
CCACTGGTAT TGGCASTTGG AGGAAGAGTG CGCAACGAG CCCAGCCAAG CTGTCTCAGT 1440  
GACAAACCCA TACCCTTGTG AAGAATGGAG AAGTGTGGAG GACTTCCAGG GAGGAAATAA 1500

- 81 -

	AATTGCCGTT AATAAAAATC AATTTGCTCT AATTGAAGGA AAAAACAAAA CTGTAAGTAC	1560
5	CCTTGTTATC CAAGCGGCAA ATGTGTCAGC TTTGTACAAA TGTGAAGCGG TCAACAAAGT	1620
	CGGGAGAGGA GAGAGGGTGA TCTCCTTCCA CGTGACCAGG GGTCTGAAA TTACTTTGCA	1680
	ACCTGACATG CAGCCCACTG AGCAGGAGAG CGTGTCTTTG TGGTCACTG CAGACAGATC	1740
10	TACGTTTGAG AACCTCACAT GGTACAAGCT TGGCCACAG CCTCTGCCAA TCCATGTGGG	1800
	AGAGTTGCCC ACACCTGTTT GCAAGAACTT GGATACTCTT TGGAAATTGA ATGCCACCAT	1860
15	GTCTCTAAT AGCACAAATG ACATTTTGAT CATGGAGCTT AAGAATGCAT CCTGCAGGA	1920
	CCAAGGAGAC TATGTCTGCC TTGCTCAAGA CAGGAAGACC AAGAAAAGAC ATTGCGTGGT	1980
	CAGGCAGCTC ACAGTCTAG AGCGTGTGGC ACCCAGGATC ACAGGAAACC TGGAGAATCA	2040
20	GACGACAAGT ATTGGGGAAA GCATCGAAGT CTCATGCACG GCATCTGGGA ATCCCCCTCC	2100
	ACAGATCATG TGGTTTAAAG ATAATGAGAC CCTGTAGAA GACTCAGGCA TTGTATTGAA	2160
25	GGATGGGAAC CGGAACCTCA CTATCCGAG AGTGAGGAAG GAGGACGAAG GCCTCTACAC	2220
	CTGCCAGGCA TGCAGTGTTC TTGGCTGTGC AAAAGTGGAG GCATTTTCA TAATAGAAGG	2280
	TGCCCAGGAA AAGACGAACT TGGAAATCAT TATTCTAGTA GGCACGACGG TGATTGCCAT	2340
30	GTCTCTCTGG CTACTTCTTG TCATCATCCT AGGGACCGTT TAA	2383

- 82 -

WHAT IS CLAIMED IS:

1. A soluble VEGF inhibitor in substantially pure form  
5 which specifically binds VEGF and inhibits cellular VEGF  
receptor activity.

2. The soluble VEGF inhibitor according to Claim 1  
wherein the soluble VEGF receptor is selected from the  
10 group consisting of sVEGF-RI, sVEGF-RII, sVEGF-RTMI and  
sVEGF-RTMII.

3. The soluble VEGF inhibitor of Claim 2 corresponding  
to sVEGF-RI comprising the amino acid sequence:  
15

Met Val Ser Tyr Trp Asp Thr Gly Val Leu Leu

Cys Ala Leu Leu Ser Cys Leu Leu Leu Thr Gly Ser Ser Ser Gly

20 Ser Lys Leu Lys Asp Pro Glu Leu Ser Leu Lys Gly Thr Gln His

Ile Met Gln Ala Gly Gln Thr Leu His Leu Gln Cys Arg Gly Glu

25 Ala Ala His Lys Trp Ser Leu Pro Glu Met Val Ser Lys Glu Ser

Glu Arg Leu Ser Ile Thr Lys Ser Ala Cys Gly Arg Asn Gly Lys

30

- 83 -

Gln Phe Cys Ser Thr Leu Thr Leu Asn Thr Ala Gln Ala Asn His  
5 Thr Gly Phe Tyr Ser Cys Lys Tyr Leu Ala Val Pro Thr Ser Lys  
Lys Lys Glu Thr Glu Ser Ala Ile Tyr Ile Phe Ile Ser Asp Thr  
Gly Arg Pro Phe Val Glu Met Tyr Ser Glu Ile Pro Glu Ile Ile  
10 His Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val Thr  
Ser Pro Asn Ile Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr  
Leu Ile Pro Asp Gly Lys Arg Ile Ile Trp Asp Ser Arg Lys Gly  
15 Phe Ile Ile Ser Asn Ala Thr Tyr Lys Glu Ile Gly Leu Leu Thr  
Cys Glu Ala Thr Val Asn Gly His Leu Tyr Lys Thr Asn Tyr Leu  
20 Thr His Arg Gln Thr Asn Thr Ile Ile Asp Val Gln Ile Ser Thr

25

30

- 84 -

Pro Arg Pro Val Lys Leu Leu Arg Gly His Thr Leu Val Leu Asn  
Cys Thr Ala Thr Thr Pro Leu Asn Thr Arg Val Gln Met Thr Trp  
5 Ser Tyr Pro Asp Glu Lys Asn Lys Arg Ala Ser Val Arg Arg Arg  
Ile Asp Gln Ser Asn Ser His Ala Asn Ile Phe Tyr Ser Val Leu  
10 Thr Ile Asp Lys Met Gln Asn Lys Asp Lys Gly Leu Tyr Thr Cys  
Arg Val Arg Ser Gly Pro Ser Phe Lys Ser Val Asn Thr Ser Val  
His Ile Tyr Asp Lys Ala Phe Ile Thr Val Lys His Arg Lys Gln  
15 Gln Val Leu Glu Thr Val Ala Gly Lys Arg Ser Tyr Arg Leu Ser  
Met Lys Val Lys Ala Phe Pro Ser Pro Glu Val Val Trp Leu Lys  
20 Asp Gly Leu Pro Ala Thr Glu Lys Ser Ala Arg Tyr Leu Thr Arg

25

30

- 85 -

Gly Tyr Ser Leu Ile Ile Lys Asp Val Thr Glu Glu Asp Ala Gly  
Asn Tyr Thr Ile Leu Leu Ser Ile Lys Gln Ser Asn Val Phe Lys  
5 Asn Leu Thr Ala Thr Leu Ile Val Asn Val Lys Pro Gln Ile Tyr  
Glu Lys Ala Val Ser Ser Phe Pro Asp Pro Ala Leu Tyr Pro Leu  
10 Gly Ser Arg Gln Ile Leu Thr Cys Thr Ala Tyr Gly Ile Pro Gln  
Pro Thr Ile Lys Trp Phe Trp His Pro Cys Asn His Asn His Ser  
Glu Ala Arg Cys Asp Phe Cys Ser Asn Asn Glu Glu Ser Phe Ile  
15 Leu Asp Ala Asp Ser Asn Met Gly Asn Arg Ile Glu Ser Ile Thr  
Gln Arg Met Ala Ile Ile Glu Gly Lys Asn Lys Met Ala Ser Thr  
20 Leu Val Val Ala Asp Ser Arg Ile Ser Gly Ile Tyr Ile Cys Ile

25

30

- 86 -

Ala Ser Asn Lys Val Gly Thr Val Gly Arg Asn Ile Ser Phe Tyr  
Ile Thr Asp Val Pro Asn Gly Phe His Val Asn Leu Glu Lys Met  
5 Pro Thr Glu Gly Glu Asp Leu Lys Leu Ser Cys Thr Val Asn Lys  
Phe Leu Tyr Arg Asp Val Thr Trp Ile Leu Leu Arg Thr Val Asn  
10 Asn Arg Thr Met His Tyr Ser Ile Ser Lys Gln Lys Met Ala Ile  
Thr Lys Glu His Ser Ile Thr Leu Asn Leu Thr Ile Met Asn Val  
Ser Leu Gln Asp Ser Gly Thr Tyr Ala Cys Arg Ala Arg Asn Val  
15 Tyr Thr Gly Glu Glu Ile Leu Gln Lys Lys Glu Ile Thr Ile Arg  
Gly Glu His Cys Asn Lys Lys Ala Val Phe Ser Arg Ile Ser Lys  
20 Phe Lys Ser Thr Arg Asn Asp Cys Thr Thr Gln Ser Asn Val Lys  
His. (SEQ. ID. NO.: 6)

25

30



- 87 -

4. The soluble VEGF inhibitor of Claim 2 corresponding to sVEGF-RI comprising the amino acid sequence:

5       Ser Lys Leu Lys Asp Pro Glu Leu Ser Leu Lys Gly Thr Gln His  
Ile Met Gln Ala Gly Gln Thr Leu His Leu Gln Cys Arg Gly Glu  
Ala Ala His Lys Trp Ser Leu Pro Glu Met Val Ser Lys Glu Ser  
10       Glu Arg Leu Ser Ile Thr Lys Ser Ala Cys Gly Arg Asn Gly Lys  
Gln Phe Cys Ser Thr Leu Thr Leu Asn Thr Ala Gln Ala Asn His  
15       Thr Gly Phe Tyr Ser Cys Lys Tyr Leu Ala Val Pro Thr Ser Lys  
Lys Lys Glu Thr Glu Ser Ala Ile Tyr Ile Phe Ile Ser Asp Thr  
Gly Arg Pro Phe Val Glu Met Tyr Ser Glu Ile Pro Glu Ile Ile  
20       His Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val Thr

25

30

- 88 -

Ser Pro Asn Ile Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr  
Leu Ile Pro Asp Gly Lys Arg Ile Ile Trp Asp Ser Arg Lys Gly  
5 Phe Ile Ile Ser Asn Ala Thr Tyr Lys Glu Ile Gly Leu Leu Thr  
Cys Glu Ala Thr Val Asn Gly His Leu Tyr Lys Thr Asn Tyr Leu  
10 Thr His Arg Gln Thr Asn Thr Ile Ile Asp Val Gln Ile Ser Thr  
Pro Arg Pro Val Lys Leu Leu Arg Gly His Thr Leu Val Leu Asn  
Cys Thr Ala Thr Thr Pro Leu Asn Thr Arg Val Gln Met Thr Trp  
15 Ser Tyr Pro Asp Glu Lys Asn Lys Arg Ala Ser Val Arg Arg Arg  
Ile Asp Gln Ser Asn Ser His Ala Asn Ile Phe Tyr Ser Val Leu  
20 Thr Ile Asp Lys Met Gln Asn Lys Asp Lys Gly Leu Tyr Thr Cys

25

30

- 89 -

Arg Val Arg Ser Gly Pro Ser Phe Lys Ser Val Asn Thr Ser Val  
His Ile Tyr Asp Lys Ala Phe Ile Thr Val Lys His Arg Lys Gln  
5 Gln Val Leu Glu Thr Val Ala Gly Lys Arg Ser Tyr Arg Leu Ser  
Met Lys Val Lys Ala Phe Pro Ser Pro Glu Val Val Trp Leu Lys  
10 Asp Gly Leu Pro Ala Thr Glu Lys Ser Ala Arg Tyr Leu Thr Arg  
Gly Tyr Ser Leu Ile Ile Lys Asp Val Thr Glu Glu Asp Ala Gly  
Asn Tyr Thr Ile Leu Leu Ser Ile Lys Gln Ser Asn Val Phe Lys  
15 Asn Leu Thr Ala Thr Leu Ile Val Asn Val Lys Pro Gln Ile Tyr  
Glu Lys Ala Val Ser Ser Phe Pro Asp Pro Ala Leu Tyr Pro Leu  
20 Gly Ser Arg Gln Ile Leu Thr Cys Thr Ala Tyr Gly Ile Pro Gln

25

30

- 90 -

Pro Thr Ile Lys Trp Phe Trp His Pro Cys Asn His Asn His Ser  
Glu Ala Arg Cys Asp Phe Cys Ser Asn Asn Glu Glu Ser Phe Ile  
5 Leu Asp Ala Asp Ser Asn Met Gly Asn Arg Ile Glu Ser Ile Thr  
Gln Arg Met Ala Ile Ile Glu Gly Lys Asn Lys Met Ala Ser Thr  
10 Leu Val Val Ala Asp Ser Arg Ile Ser Gly Ile Tyr Ile Cys Ile  
Ala Ser Asn Lys Val Gly Thr Val Gly Arg Asn Ile Ser Phe Tyr  
Ile Thr Asp Val Pro Asn Gly Phe His Val Asn Leu Glu Lys Met  
15 Pro Thr Glu Gly Glu Asp Leu Lys Leu Ser Cys Thr Val Asn Lys  
Phe Leu Tyr Arg Asp Val Thr Trp Ile Leu Leu Arg Thr Val Asn  
20 Asn Arg Thr Met His Tyr Ser Ile Ser Lys Gln Lys Met Ala Ile

25

30

- 91 -

Thr Lys Glu His Ser Ile Thr Leu Asn Leu Thr Ile Met Asn Val

Ser Leu Gln Asp Ser Gly Thr Tyr Ala Cys Arg Ala Arg Asn Val

5

Tyr Thr Gly Glu Glu Ile Leu Gln Lys Lys Glu Ile Thr Ile Arg

Gly Glu His Cys Asn Lys Lys Ala Val Phe Ser Arg Ile Ser Lys

10

Phe Lys Ser Thr Arg Asn Asp Cys Thr Thr Gln Ser Asn Val Lys

His. (SEQ. ID. NO.: 12)

5. The soluble VEGF inhibitor of Claim 2 corresponding  
15 to sVEGF-RII comprising the amino acid sequence:

MQSKVLLAVALWLCVETRAASVGLPSVSLDLPRLSIQKDILTIKANTTLQITCRGQR  
DLDWLWPNNQSGSEQRVEVTECSDGLFCKTLTIPKVIGNDTGAYKCFYRETDLASVI  
YVYVQDYRSPFIASVSDQHGVVYITENKNTVVIPCLGSISNLNVSLCARYPEKRFV  
20 PDGNRISWDSKKGFTIPSYMISYAGMVFCFAKINDESYQSIMYIVVVVGYRIYDVVL  
SPSHGIELSVGEKLVNCTARTELVNVDNFWEYPSKQHKLVNRDLKTQSGSEM  
KKFLSTLTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRVHEKPFVAFGSGMESLVEA  
TVGERVRIPAKYLGYPPEIKWYKNGIPLESNHTIKAGHVLTIMEVSEKDTGNYTVI  
LTNPISKEKQSHVSVLVVYVPPQIGEKSLISPVDSYQYGTQTTLCTVYAIPPPHEI  
25 HWYWQLEEECANEPSQAVSVTNYPCEEWSVEDFQGGNKIAVNKNQFALIEGKNKT  
VSTLVIQAANVSALYKCEAVNKVGRGERVISFEVTRGPEITLQPDMPTEQESVSLW  
CTADRSTFENLTWYKLGPPQLPIHVGELEPTPVCKNLDLWKLNATMFSNSTNDILIM  
ELKNASLQDQGDYVCLAQDRKTKRHCVVRLTVLER. (SEQ.ID.NO.: 13)

30

- 92 -

6. The soluble VEGF inhibitor of Claim 2 corresponding to sVEGF-RTMI comprising the amino acid sequence:

5 MVSYWDTGVLLCALLSCLLLTGSSSSGSKLKDPELSLKGTQHIMQAGQTLHLQCRGEA  
AHKWSLPENVSKESERLSITKSACGRNGKQFCSTLTNLTAQANHTGFYSCKYLAVPT  
SKKETESAIYIFISDTGRPFVEMYSEIPEIIHMTGRELVIPCRVTSPNITVTLKK  
FPLDTLIPDGKRIIWDNRKGFIIISNATYKEIGLLTCEATVNGHLYKTNLTHRQTNT  
IIDVQISTPRPVKLLRGHTLVNLCTATTPLNTRVQMTWSYPDEKNKRASVRRRIDQS  
10 NSHANIFYSVLTIDKMQNKDKGLYTCRVRSGPSFKSVNTSVHIYDKAFITVKHRKQQ  
VLETVAGKRSYRLSMKVKAFFPSPEVVWLKDGLPATEKSARYLTRGYSLLIKDVTEED  
AGNYTILLSIKQSNVFNLTATLIVNVKPKIYEKAVSSFDPALYPLGSRQILTCTA  
YGIPQPTIKWFHPCNHNHSEARCDFCNNNEESFILDADSNMGNRIESITQMAIIE  
GKNKMASTLVVADSRISGIYICIASNKVGTVGRNISFYITDVPNGFHVNLKMPTEG  
15 EDLKLSTVKNFLYRDVTWILLRTVNNRTMHYSISKQKMAITKEHSITLNLTIMNVS  
LQDSGTIACRARNVYTGEIILQKKEITIRDQEAPYLLRNLSDHVTVAISSSTTLDDCHA  
NGVPEPQITWFKNNHKIQEPGIILGPGSSTLFIERVTEEDGVYHCKATNQKGSVE  
SSAYLTVQGTSDKSNLELITLTCTCVAATLFWLLLTLLI. (SEQ. ID. NO.:  
14)

20

7. The soluble VEGF inhibitor of Claim 2 corresponding to sVEGF-RTMII comprising the amino acid sequence:

MQSKVLLAVALWLCVETRAASVGLPSVSLDLPRLSIQKDILTIIKANTTLQITCRGQR  
25 DLDWLWPNNQSGSEQRVEVTECSDFLCKTLTIPKVIGNDTGAYKCFYRETDLASVI  
YVYVQDYRSPFIASVSDQHGVYIITENKNKTVPCLGSISNLNVSLCARYPEKRFV  
PDGNRISWDSKKGFTIPSYMISYAGMVFEAKINDESYQSIMYIVVVVGYRIYDVVL  
SPSHGIELSVGEKLVNLCTARTELNVGIDFNWEYPSKQHKLVNRDLKTQSGSEM  
KKFLSTLTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRVHEKPFVAFSGMESLVEA  
30 TVGERVRIPAKYLGYPPEIKWYKNGIPLESNETIKAGHVLTIMEVSRDTGNYTVI  
LTNPISKEKQSHVVSLLVYVPPQIGEKSLISPVDSTQYGTQTTLTCTVYAIPPPHHI

- 93 -

HWYWQLEEECANEPSQAVSVTNPYPCEEWRSVEDFQGGNKIAVNKNQFALIEGKNKT  
VSTLVIQAANVSALYKCEAVNKVGRGERVISFHVTRGPEITLQPDMPTEQESVSLW  
CTADRSTFENLTWYKLGPOPLPIHVGE LPTPVCKNLDLWKLNATMFSNSTNDILIM  
5 ELKNASLQDQGDYVCLAQDRKTKKRHCVVVRQLTVLERVAPTITGNLENQTTSIGESI  
EVSCTASGNPPPQIMWFKDNETLVEDSGIVLKDGNRNLTI RRV RKEDEGLYCQACSV  
LGCAKVEAFFIIEGAQEKTNLEIIILVGTTVIAMFFWLLLVIILGTV. (SEQ.  
ID. NO.: 15)

10 8. An expression vector comprising a promoter, and a  
DNA sequence encoding a soluble VEGF inhibitor for  
expression in recombinant host cells wherein the soluble  
VEGF inhibitor is selected from the group consisting of  
sVEGF-RI, sVEGF-RII, sVEGF-RTMI and sVEGF-RTMII.  
15

9. The expression vector of Claim 8 wherein the DNA  
encoding the sVEGF-RI comprises the nucleotide sequence:

20 GCGGACACTCCTCTCGGCTCCTCCCCGCGAGCGCGCGGCTCGGACCGGGCTCCGGGG  
CTCGGGTGCAGCGGCCAGCGGGCTGGCGGCGAGGATTACCGGGGAAGTGGTTGTCTC  
CTGGCTGGAGCCGCCAGACGGGCGCTCAGGGCCGGGGCCGGCGGGCGGAACGAGAGG  
25 ACGGACTCTGGCGGCCGGGTGCTTGGCCGGGGCAGCGCGGCCACCGGGCGAGCAGGCCG  
30

- 94 -

CGTCGGGCTCACC ATG GTC AGC TAC TGG GAC ACC GGG GTC CTG CTG  
TGC GCG CTG CTC AGC TGT CTG CTT CTC ACA GGA TCT AGT TCA GGT  
5 TCA AAA TTA AAA GAT CCT GAA CTG AGT TTA AAA GGC ACC CAG CAC  
ATC ATG CAA GCA GGC CAG ACA CTG CAT CTC CAA TGC AGG GGG GAA  
10 GCA GCC CAT AAA TGG TCT TTG CCT GAA ATG GTG AGT AAG GAA AGC  
GAA AGG CTG AGC ATA ACT AAA TCT GCC TGT GGA AGA AAT GCC AAA  
CAA TTC TGC AGT ACT TTA ACC TTG AAC ACA GCT CAA GCA AAC CAC  
15 ACT GGC TTC TAC AGC TGC AAA TAT CTA GCT GTA CCT ACT TCA AAG  
AAG AAG GAA ACA GAA TCT CCA ATC TAT ATA TTT ATT ACT GAT ACA  
20 GGT AGA CCT TTC GTA GAG ATG TAC AGT GAA ATC CCC GAA ATT ATA

25

30



- 95 -

CAC ATG ACT GAA GGA AGG GAG CTC GTC ATT CCC TGC CGG GTT ACG  
TCA CCT AAC ATC ACT GTT ACT TTA AAA AAG TTT CCA CTT GAC ACT  
5 TTG ATC CCT GAT GGA AAA CGC ATA ATC TGG GAC AGT AGA AAG GGC  
TTC ATC ATA TCA AAT GCA ACG TAC AAA GAA ATA GGG CTT CTG ACC  
10 TGT GAA GCA ACA GTC AAT GGG CAT TTG TAT AAG ACA AAC TAT CTC  
ACA CAT CGA CAA ACC AAT ACA ATC ATA GAT GTC CAA ATA AGC ACA  
CCA CGC CCA GTC AAA TTA CTT AGA GGC CAT ACT CTT GTC CTC AAT  
15 TGT ACT GCT ACC ACT CCC TTG AAC ACG AGA GTT CAA ATG ACC TGG  
AGT TAC CCT GAT GAA AAA AAT AAG AGA GCT TCC GTA AGG CGA CGA  
20 ATT GAC CAA AGC AAT TCC CAT GCC AAC ATA TTC TAC AGT GTT CTT

25

30

- 96 -

ACT ATT GAC AAA ATG CAG AAC AAA GAC AAA GGA CTT TAT ACT TGT  
CGT GTA AGG AGT GGA CCA TCA TTC AAA TCT GTT AAC ACC TCA GTG  
5 CAT ATA TAT GAT AAA GCA TTC ATC ACT GTG AAA CAT CGA AAA CAG  
CAG GTG CTT GAA ACC GTA GCT GGC AAG CGG TCT TAC CGG CTC TCT  
10 ATG AAA GTG AAG GCA TTT CCC TCG CCG GAA GTT GTA TGG TTA AAA  
GAT GGG TTA CCT GCG ACT GAG AAA TCT GCT CGC TAT TTG ACT CGT  
GGC TAC TCG TTA ATT ATC AAG GAC GTA ACT GAA GAG GAT GCA GGG  
15 AAT TAT ACA ATC TTG CTG AGC ATA AAA CAG TCA AAT GTG TTT AAA  
AAC CTC ACT GCC ACT CTA ATT GTC AAT GTG AAA CCC CAG ATT TAC  
20 GAA AAG GCC GTG TCA TCG TTT CCA GAC CCG GCT CTC TAC CCA CTG

25

30

- 97 -

GGC AGC AGA CAA ATC CTG ACT TGT ACC GCA TAT GGT ATC CCT CAA  
CCT ACA ATC AAG TGG TTC TGG CAC CCC TGT AAC CAT AAT CAT TCC  
5 GAA GCA AGG TGT GAC TTT TGT TCC AAT AAT GAA GAG TCC TTT ATC  
CTG GAT GCT GAC AGC AAC ATG GGA AAC AGA ATT GAG AGC ATC ACT  
10 CAG CGC ATG GCA ATA ATA GAA GGA AAG AAT AAG ATG GCT AGC ACC  
TTG GTT GTG GCT GAC TCT AGA ATT TCT GGA ATC TAC ATT TGC ATA  
GCT TCC AAT AAA GTT GGG ACT GTG GGA AGA AAC ATA AGC TTT TAT  
15 ATC ACA GAT GTG CCA AAT GGG TTT CAT GTT AAC TTG GAA AAA ATG  
CCG ACG GAA GGA GAG GAC CTG AAA CTG TCT TGC ACA GTT AAC AAG  
20 TTC TTA TAC AGA GAC GTT ACT TGG ATT TTA CTG CCG ACA GTT AAT

25

30

- 98 -

AAC AGA ACA ATG CAC TAC AGT ATT AGC AAG CAA AAA ATG GCC ATC  
ACT AAG GAG CAC TCC ATC ACT CTT AAT CTT ACC ATC ATG AAT GTT  
5 TCC CTG CAA GAT TCA GGC ACC TAT GCC TGC AGA GCC AGG AAT GTA  
TAC ACA GGG GAA GAA ATC CTC CAG AAG AAA GAA ATT ACA ATC AGA  
10 GGT GAG CAC TGC AAC AAA AAG GCT GTT TTC TCT CGG ATC TCC AAA  
TTT AAA AGC ACA AGG AAT GAT TGT ACC ACA CAA AGT AAT GTA AAA  
CAT TAA AGGACTCATTAAAAAGTAACAGTTGTCTCATATCATCTTGATTTATTGTCA  
15 CTGTTGCTAACTTTTCAGGCTCGGAGGAGATGCTCCTCCCAAATGAGTTGGGAGATGAT  
AGCAGTAATAATGAGACCCCCGGGCTCCAGCTCTGGGCCCCCATTCAGGCCGAGGGG

20

25

30

- 99 -

CTGCTCCGGGGGGCCCACTTGGTGACGTTTGGATTGGAGGATCCCTGCACTGCCTTC  
TCTGTGTTTGTGCTCTTGCTGTTTTCTCCTGCCTGATAAACAACAACCTGGGATGATC  
CTTTCCATTTTATGATGCCAACCTCTTTTTATTTTTAAGCGGCGCCCTATAGT.

5 (SEQ. ID. NO.: 5)

10. The expression vector of Claim 8 wherein the DNA  
encoding the sVEGF-RII comprises the nucleotide  
sequence:

10

GGTGTGGTCGCTGCGTTTCTCTGCCTGCGCCGGGCATCACTTGCGCGCCGCAGAA  
AGTCCGTCTGGCAGCCTGGATATCCTCTCCTACCGGCACCCGCAGACGCCCCCTGCA  
GCCGCGGTGCGCGCCCGGGCTCCCTAGCCCTGTGCGCTCAACTGTCTGCGCTGCG  
GGGTGCCGCGAGTTCCACCTCCGCGCCTCCTTCTCTAGACAGGCGCTGGGAGAAAG  
15 AACCGGCTCCCGAGTTCGGGCATTTCGCCCCGGCTCGAGGTGCAGGATGCAGAGCAA  
GGTGTGCTGCGCCGTGCCCCCTGTGGCTCTGCGTGGAGACCCGGGCGCCTCTGTGG  
GTTTGCTAGTGTTCCTCTTGATCTGCCCAGGCTCAGCATACAAAAAGACATACTT  
ACAATTAAGGCTAATACAACCTCTTCAAATTACTTGCAGGGGACAGAGGGACTTGGA  
CTGGCTTTGGCCCAATAATCAGAGTGGCAGTGAGCAAAGGGTGGAGGTGACTGAGT  
20 GCAGCGATGGCCTCTTCTGTAAAGACACTCACAATTCCAAAAGTGATCGGAAATGAC  
ACTGGAGCCTACAAGTGCTTCTACCGGGAACTGACTTGGCCTCGGTCAATTTATGT  
CTATGTTCAAGATTACAGATCTCCATTTATTGCTTCTGTAGTGACCAACATGGAG  
TCGTGTACATTACTGAGAACAAAAACAACTGTGGTGATTCCATGTCTCGGGTCC  
ATTTCAAATCTCAACGTGTCACTTTGTGCAAGATACCCAGAAAAGAGATTTGTTCC  
25 TGATGGTAACAGAATTTCTGGGACAGCAAGAAGGGCTTTACTATTCCAGCTACA  
TGATCAGCTATGCTGGCATGGTCTTCTGTGAAGCAAAAATTAATGATGAAAGTTAC  
CAGTCTATTATGTACATAGTTGTCGTTGTAGGGTATAGGATTTATGATGTGGTTCT  
GAGTCCGTCTCATGGAATTGAACTATCTGTTGGAGAAAAGCTTGTCTTAAATTGTA

30

- 100 -

CAGCAAGAACTGAACTAAATGTGGGGATTGACTTCAACTGGGAATACCCCTTCTTCG  
AAGCATCAGCATAAGAACTTGTAACCGAGACCTAAAAACCCAGTCTGGGAGTGA  
GATGAAGAAATTTTTGAGCACCTTAACTATAGATGGTGTAACCCGGAGTGACCAAG  
5 GATTGTACACCTGTGCAGCATCCAGTGGGCTGATGACCAAGAAGAACAGCACATTT  
GTCAGGGTCCATGAAAAACCTTTTGTGCTTTTGGAAAGTGGCATGGAATCTCTGGT  
GGAAGCCACGGTGGGGGAGCGTGTGAGAATCCCTGCCAAGTACCTTGGTTACCCAC  
CCCCAGAAATAAAATGGTATAAAAAATGGAATACCCCTTGAGTCCAATCACACAATT  
AAAGCGGGGCATGTACTGACGATTATGGAAGTGAGTGAAAGAGACACAGGAAATTA  
10 CACTGTATCCTTACCAATCCCATTTCAAAGGAGAAGCAGAGCCATGTGGTCTCTC  
TGTTGTGTATGTCCACCCAGATTGGTGAGAAATCTCTAATCTCTCTCTGTGGAT  
TCCTACCAGTACGGCACCCTCAAACGCTGACATGTACGGTCTATGCCATTCTCTCC  
CCCGCATCACATCCACTGGTATTGGCAGTTGGAGGAAGAGTGCGCCAACGAGCCCA  
GCCAAGCTGTCTCAGTGACAAACCCATACCCTTGTGAAGAATGGAGAAGTGTGGAG  
15 GACTTCCAGGGAGGAAATAAAATTGCCGTTAATAAAAAATCAATTTGCTCTAATTGA  
AGGAAAAAACAAACTGTAAGTACCCCTTGTATCCAAGCGGCAAATGTGTGAGCTT  
TGTAACAATGTGAAGCGGTCAACAAAGTCGGGAGAGGAGAGAGGGTGATCTCCTTC  
CACGTGACCAGGGGTCCTGAAATTACTTTGCAACCTGACATGCAGCCCACTGAGCA  
GGAGAGCGTGTCTTTGTGGTGCACTGCAGACAGATCTACGTTTGAGAACCTCACAT  
20 GGTACAAGCTTGGCCACAGCCTCTGCCAATCCATGTGGGAGAGTTGCCACACCT  
GTTTGCAAGAACTTGGATACTCTTTGGAAATTGAATGCCACCATGTTCTCTAATAG  
CACAAATGACATTTTGATCATGGAGCTTAAGAATGCATCCTTGCAGGACCAAGGAG  
ACTATGTCTGCCTTGCTCAAGACAGGAAGACCAAGAAAAGACATTGCGTGGTCAGG  
CAGCTCACAGTCCTAGAGCGTTAA. (SEQ. ID. NO.: 16)

25

11. The expression vector of Claim 8 wherein the DNA encoding the sVEGF-RTM1 comprises the nucleotide sequence:

30 GCGCTCACCATGGTCAGCTACTGGGACACCGGGGTCCTGCTGTGCGCGCTGCTCAG  
CTGCTGCTTCTCACAGGATCTAGTTCAGGTTCAAAATTAAAAGATCCTGAACTGA

- 101 -

GTTTAAAAGGCACCCAGCACATCATGCAAGCAGGCCAGACACTGCATCTCCAATGC  
AGGGGGGAAGCAGCCCATAAATGGTCTTTGCCTGAAATGGTGAGTAAGGAAAGCGA  
AAGGCTGAGCATAACTAAATCTGCCTGTGGAAGAAATGGCAAACAATTCTGCAGTA  
5 CTTTAACCTTGAACACAGCTCAAGCAAACCACACTGGCTTCTACAGCTGCAAATAT  
CTAGCTGTACCTACTTCAAAGAAGAAGGAAACAGAATCTGCAATCTATATATTTAT  
TAGTGATACAGGTAGACCTTTTCGTAGAGATGTACAGTGAAATCCCCGAAATTATAC  
ACATGACTGAAGGAAGGGAGCTCGTCATTCCCTGCCGGGTACGTCACCTAACATC  
ACTGTTACTTTAAAAAAGTTTCCACTTGACACTTTGATCCCTGATGGAAAACGCAT  
10 AATCTGGGACAGTAGAAAGGGCTTCATCATATCAAATGCAACGTACAAAGAAATAG  
GGCTTCTGACCTGTGAAGCAACAGTCAATGGGCATTTGTATAAGACAACTATCTC  
ACACATCGACAAACCAATACAATCATAGATGTCCAAATAAGCACACCACGCCCAGT  
CAAATTACTTAGAGGCCATACTCTTGTCCTCAATTGTACTGCTACCACTCCCTTGA  
ACACGAGAGTTCAAATGACCTGGAGTTACCCTGATGAAAAAATAAGAGAGCTTCC  
15 GTAAGGCGACGAATTGACCAAAGCAATTCCCATGCCAACATATTCTACAGTGTTCT  
TACTATTGACAAATGCAGAACAAAGACAAAGGACTTTATACTTGTCGTGTAAGGA  
GTGGACCATCATTCAAATCTGTTAACACCTCAGTGCATATATATGATAAAGCATT  
ATCACTGTGAAACATCGAAAACAGCAGGTGCTTGAAACCGTAGCTGGCAAGCGGTC  
TTACCGGCTCTCTATGAAAGTGAAGGCATTTCCCTCGCCGGAAGTTGTATGGTTAA  
20 AAGATGGGTTACCTGCGACTGAGAAATCTGCTCGCTATTTGACTCGTGGCTACTCG  
TTAATTATCAAGGACGTAAGTGAAGAGGATGCAGGGAATTATACAATCTTGCTGAG  
CATAAACAGTCAAATGTGTTTAAAAACCTCACTGCCACTCTAATTGTCAATGTGA  
AACCCAGATTTACGAAAAGGCCGTGTCATCGTTTCCAGACCCGGCTCTCTACCCA  
CTGGGCAGCAGACAAATCCTGACTTGTACCGCATATGGTATCCCTCAACCTACAAT  
25 CAAGTGGTTCTGGCACCCCTGTAACCATAATCATTCCGAAGCAAGGTGTGACTTTT  
GTTCCAATAATGAAGAGTCCTTTATCCTGGATGCTGACAGCAACATGGGAAACAGA  
ATTGAGAGCATCACTCAGCGCATGGCAATAATAGAAGGAAAGAATAAGATGGCTAG  
CACCTTGGTTGTGGCTGACTCTAGAATTTCTGGAATCTACATTTGCATAGCTTCCA  
ATAAAGTTGGGACTGTGGGAAGAAACATAAGCTTTTATATCACAGATGTGCCAAAT  
30 GGGTTTCATGTTAACTTGGAAGAAATGCCGACGGAAGGAGAGGACCTGAAACTGTC  
TTGCACAGTTAACAAGTTCTTATACAGAGACGTTACTTGGATTTTACTGCGGACAG

- 102 -

TTAATAACAGAACAATGCACTACAGTATTAGCAAGCAAAAAATGGCCATCACTAAG  
GAGCACTCCATCACTCTTAATCTTACCATCATGAATGTTTCCCTGCAAGATTCAGG  
CACCTATGCCTGCAGAGCCAGGAATGTATACACAGGGGAAGAAATCCTCCAGAAGA  
5 AAGAAATTACAATCAGAGATCAGGAAGCACCATACCTCCTGCGAAACCTCAGTGAT  
CACACAGTGGCCATCAGCAGTTCCACCACCTTTAGACTGTCATGCTAATGGTGTCCC  
CGAGCCTCAGATCACTTGGTTTAAAAACAACCACAAAATACAACAAGAGCCTGGAA  
TTATTTTAGGACCAGGAAGCAGCAGCTGTTTATTGAAAGAGTCACAGAAGAGGAT  
GAAGGTGTCTATCACTGCAAAGCCACCAACCAGAAGGGCTCTGTGGAAAGTTCAGC  
10 ATACCTCACTGTTCAAGGAACCTCGGACAAGTCTAATCTGGAGCTGATCACTCTAA  
CATGCACCTGTGTGGCTGCGACTCTCTTCTGGCTCCTATTAAACCCTCCTTATCTAA  
. (SEQ. ID. NO.: 17)

12. The expression vector of Claim 8 wherein the DNA  
15 encoding the sVEGF-RTMII comprises the nucleotide  
sequence:

CTCGAGGTGCAGGATGCAGAGCAAGGTGCTGCTGGCCGTCGCCCTGTGGCTCTGCG  
TGGAGACCCGGGCCCTCTGTGGGTTTGCCTAGTGTTTCTCTTGATCTGCCCAGG  
20 CTCAGCATACAAAAGACATACTTACAATTAAGGCTAATACAACTCTTCAAATTAC  
TTGCAGGGGACAGAGGGACTTGGACTGGCTTTGGCCCAATAATCAGAGTGGCAGTG  
AGCAAAGGGTGGAGGTGACTGAGTGCAGCGATGGCCTCTTCTGTAAGACACTCACA  
ATTCCAAAAGTGATCGGAAATGACACTGGAGCCTACAAGTGCTTCTACCGGGAAAC  
TGACTTGGCCTCGGTCAATTTATGTCTATGTTCAAGATTACAGATCTCCATTTATTG  
25 CTTCTGTTAGTGACCAACATGGAATCGTGTACATTACTGAGAACAAAAACAAAAC  
GTGGTGATTCCATGTCTCGGGTCCATTTCAAATCTCAACGTGTCACTTTGTGCAAG  
ATACCCAGAAAAGAGATTTGTTCTGATGGTAACAGAATTTCTGGGACAGCAAGA  
AGGGCTTTACTATTCCAGCTACATGATCAGCTATGCTGGCATGGTCTTCTGTGAA  
GCAAAAATTAATGATGAAAGTTACCAGTCTATTATGTACATAGTTGTGTTGTAGG  
30 GTATAGGATTTATGATGTGGTTCTGAGTCCGTCTCATGGAATTGAACTATCTGTTG  
GAGAAAAGCTTGTCTTAAATTGTACAGCAAGAACTGAACTAAATGTGGGGATTGAC



- 103 -

TTCAACTGGGAATACCCCTTCTTCGAAGCATCAGCATAAGAACTTGTAACCGAGA  
CCTAAAAACCCAGTCTGGGAGTGAGATGAAGAAATTTTTGAGCACCTTAACTATAG  
ATGGTGTAACCCGGAGTGACCAAGGATTGTACACCTGTGCAGCATCCAGTGGGCTG  
5 ATGACCAAGAAGAACAGCACATTTGTCAGGGTCCATGAAAAACCTTTTGTTGCTTT  
TGGAAGTGGCATGGAATCTCTGGTGAAGCCACGGTGGGGGAGCGTGTGAGAATCC  
CTGCGAAGTACCTTGGTTACCCACCCCCAGAAATAAAATGGTATAAAAATGGAATA  
CCCCTTGAGTCCAATCACACAATTAAGCGGGGCATGTACTGACGATTATGGAAGT  
GAGTGAAAGAGACACAGGAAATTACACTGTCTCCTTACCAATCCCATTTCAAAGG  
10 AGAAGCAGAGCCATGTGGTCTCTCTGGTTGTGTATGTCCACCCCAGATTGGTGAG  
AAATCTCTAATCTCTCTCTGTGGATTCTTACCAGTACGGCACCCTCAAACGCTGAC  
ATGTACGGTCTATGCCATTCTCTCCCCGCATCACATCCACTGGTATTGGCAGTTGG  
AGGAAGAGTGCGCCAACGAGCCCAGCCAAGCTGTCTCAGTGACAAACCCATACCTT  
TGTGAAGAATGGAGAAGTGTGGAGGACTTCCAGGGAGGAAATAAAATTGCCGTTAA  
15 TAAAAATCAATTTGCTCTAATTGAAGGAAAAACAAAACCTGTAAGTACCCTTGTTA  
TCCAAGCGGCAAATGTGTCTAGCTTTGTACAAATGTGAAGCGGTCAACAAAGTCGGG  
AGAGGAGAGAGGGTGATCTCTTCCACGTGACCAGGGGTCTGAAATTACTTTGCA  
ACCTGACATGCAGCCCACTGAGCAGGAGAGCGTGTCTTTGTGGTGCCTGACAGACA  
GATCTACGTTTGAGAACCTCACATGGTACAAGCTTGGCCACAGCCTCTGCCAATC  
20 CATGTGGGAGAGTTGCCCACACCTGTTTGCAAGAACTTGGATACTCTTTGGAAATT  
GAATGCCACCATGTTCTCTAATAGCACAAATGACATTTTGATCATGGAGCTTAAGA  
ATGCATCCTTGCCAGGACCAAGGAGACTATGTCTGCCTTGCTCAAGACAGGAAGACC  
AAGAAAAGACATTGCGTGGTCAGGCAGCTCACAGTCCTAGAGCGTGTGGCACCAC  
GATCACAGGAAACCTGGAGAATCAGACGACAAGTATTGGGGAAAGCATCGAAGTCT  
25 CATGCACGGCATCTGGGAATCCCCCTCCACAGATCATGTGGTTTAAAGATAATGAG  
ACCCCTGTAGAAGACTCAGGCATTGTATTGAAGGATGGGAACCGGAACCTCACTAT  
CCGCAGAGTGAGGAAGGAGGACGAAGGCCTCTACACCTGCCAGGCATGCAGTGTC  
TTGGCTGTGCAAAAGTGGAGGCATTTTTTCATAATAGAAGGTGCCAGGAAAAGACG  
AACTTGGAATCATTATTCTAGTAGGCACGACGGTGATTGCCATGTTCTTCTGGCT  
30 ACTTCTTGTATCATCCTAGGGACCGTTTAA. (SEQ. ID. NO.: 18)

- 104 -

13. A recombinant host cell containing the expression vector of Claim 8.

5 14. A method for inhibiting VEGF receptor function comprising the administration of the VEGF inhibitor of Claim 1 in an amount sufficient to inhibit VEGF receptor function.

10 15. The method of Claim 14 wherein the VEGF inhibitor is selected from the group consisting of sVEGF-RI, sVEGF-RII, sVEGF-RTMI, and sVEGF-RTMII.

15 16. A pharmaceutical composition comprising the inhibitor of Claim 1 and a pharmaceutically acceptable carrier.

20 17. The pharmaceutical composition of Claim 16 wherein the inhibitor is selected from the group consisting of sVEGF-RI, sVEGF-RII, sVEGF-RTMI, and sVEGF-RTMII.

25 18. A method for inhibiting angiogenesis comprising the administration of the VEGF inhibitor of Claim 1 in an amount sufficient to inhibit angiogenesis.

30

1/20

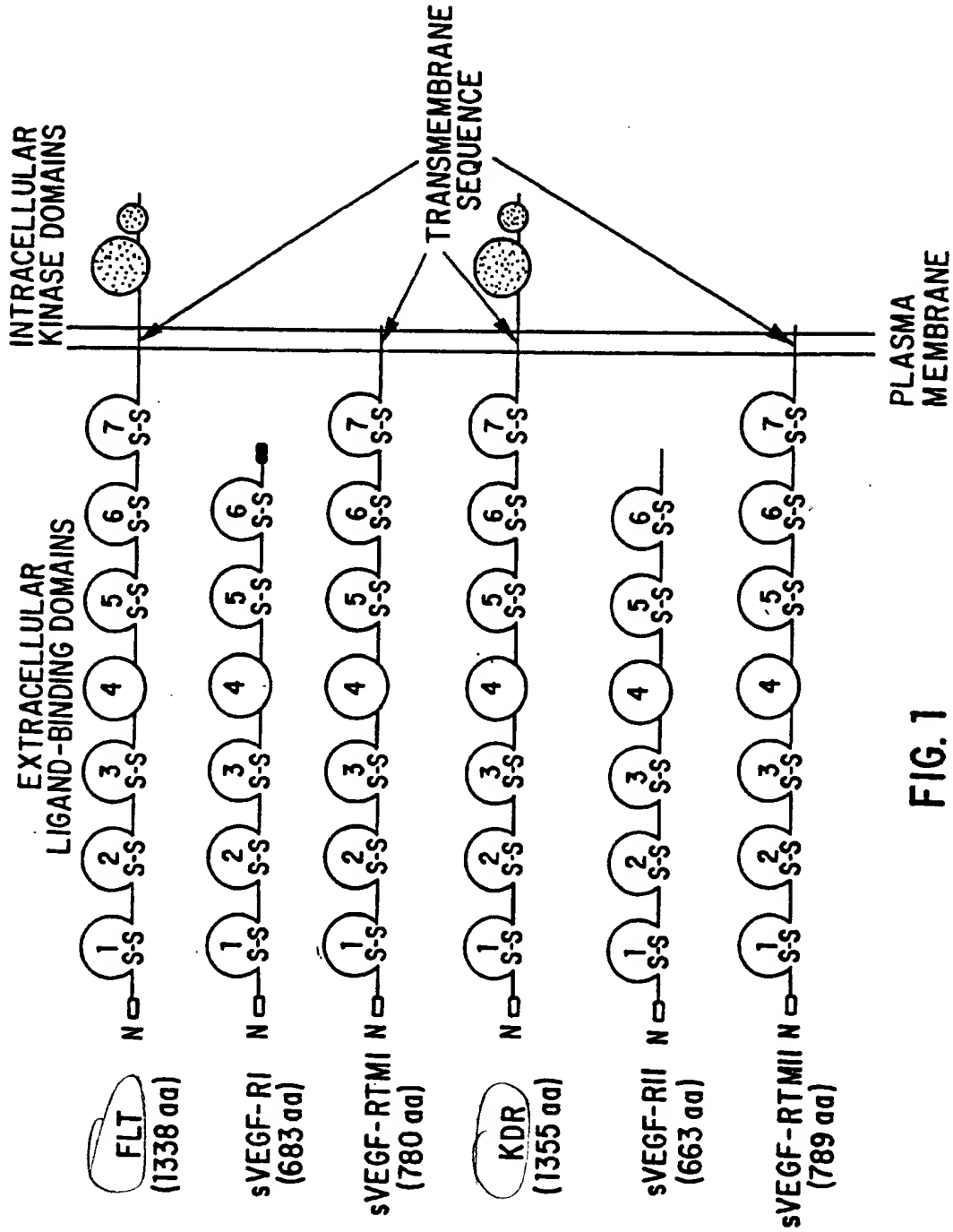


FIG. 1

2/20

GGGACACTCCTCTCGGCTCCTCCCGCAGCGGCGGCTCGGAGCGGGCTCCGGGG  
CTCGGGTGAGCGGCCAGCGGCTGGCGGAGGATTACCGGGGAAGTGGTTGTCTC  
CTGGCTGGAGCCGCGAGACGGCGCTCAGGCGGGGCGGCGGCGGCGAACGAGAG  
GACGGACTCTGGCGCGGGTCTGGCCGGGAGCGCGGCGGCGGCGGCGGAGCAGGC  
CGGTGCGGCTCACCATGCTCAGCTACTGGGACACCGGGTCTGCTGTGCGCGCTGCTC  
AGCTGTCTCTCACAGGATCTAGTTCAAGTTCAAAATTAAGATCCTGAAGTTTA  
AAGGCACCCAGCACATGCAAGCAGCGCCAGACACTGCTCCTCAATGCGGGGGAAG  
CAGCCCATAAATGGTCTTTGCCCTGAAATGGTGAGTAAGGAAAGCGAAGGCTGAGCATAACT  
AAATCTGCCCTGTGGAAGAAATGGCAACAATTTGCGAGTACTTTAACCTTGAACACAGCTCAA  
GCAACCCACACTGGCTTCTACAGCTGCAAAATATCTAGCTGTACCTACTTCAAGAAGAAGGA  
AACAGAACTCTGCAATCTATATATTTATTAGTGATACAGGTAGACCTTTCTGATAGATGTACAG  
TGAAATCCCCGAAATTATACACATGACTGAAGGAAGGAGCTCGTCAATCCCTGCCGGGTTA  
CGTCACCTAACATCACTGTACTTTAAAGTTTCCACTTGACACTTTGATCCCTGATGGAA  
AACGCATAATCTGGGACAGTAGAAGGCTCAATGGGCAATTCATATCAATGCAACGTACAAAGAAATA  
GGGCTTCTGACCTGTGAAGCAACAGTCAATGGGCAATTTGTATAGACAACTATCTCACACA  
TCGACAAACCAATACAATCATAGATGTCCAAATAGCACACACCCAGTCAAAATTAAGT  
AGGCCATACTCTTGCTCTCAATTTGCTGCTACCACTCCCTTGAACACGAGTTCAAAATGAC  
CTGGAGTTACCTGATGAATAAATAAGAGAGCTTCCGTAAAGGCGACGAAATGACCAAGCA  
ATTCCCATGCCAACAATTTCTACAGTGTCTTACTATTGACAAATGCAGAAACAAAGACAAG  
GACTTTTACTTGTGCGTGAAGGAGTGGACCATCAATCAAAATCTGTTAACACCTCAGTGCATA  
TATATGATAAAGCATTCACTGTTGAACATCGAAACAGCAGGTGCTTGAACCGTAGCT  
GGCAAGCGGTCTTACCGGCTCTCTATGAAGTGAAGGCATTTCCCTCGCCGGAAGTTGTAT

FIG. 2A

SUBSTITUTE SHEET (RULE 26)

3/20

GGTTAAAGATGGGTTACCTGCGACTGAGAAATCTGCTCGCTATTTGACTCGTGCGCTACTCG  
TTAATTATCAAGBACGTAACTGAAGAGGATGCGAGGAATTATACAATCTTGCTGAGCATAAAA  
CAGTCAATGTGTTAAACCGTCACTGCCACTCTAATTGTCAATGTGAACCCAGATTAC  
GAAAGGCGGTGTCATCGTTCCAGACCGGCTCTACCCACTGGGAGCAGACAAATCC  
TGACTTGTAACCGCATATGGTATCCCTCAACTACAATCAAGTGGTTCTGGCACCCCTGTAAAC  
CATAATCATTCGGAAGCAAGGTGTGACTTTTGTTCCAATAATGAAGAGTCCTTTATCCTGGAT  
GCTGACAGCAACATGGGAACAGAAATTGAGAGCATCACTCAGCGCATGGCAATAATAGAAG  
GAAAGAAATAGATGGCTAGCACCTTGGTTGTGGCTGACTCTAGAAATTTCTGGAATCTACATTT  
GCATAGCTTCCAATAAGTTGGGACTGTGGGAAGAAACATAAGCTTTTATATCACAGATGTG  
CCAAATGGGTTTCATGTTAACTTGGAAAAATGCCGACGGAGGAGGACCTGAACTGTC  
TTGCACAGTTAACAGTTCTTATACAGAGACGTTACTTGGATTTTACTGCGGACAGTTAATAA  
CAGAACAAATGCACATACAGTATTAGCAAGCAAAAATGGCCATCACTAAGGAGCACTCCATCA  
CTCTTAATCTTACCATCATGAATGTTTCCCTGCAAGATTGAGGACCTATGCCCTGCAGAGCCA  
GGAATGTATACACAGGGGAAGAAATCCTCCAGAAAGAAATTAACAATCAGAGGTGAGCAC  
TGCAACAAAAAGGCTGTTTCTCTCGGATCTCCAAATTTAAAGCAAGGAATGATGTACC  
ACACAAGTAATGTAAACATTAAGGACTCATTAAAGTAACAGTTGTCTCATATCATCTTG  
ATTTATTGCTCACTGTTGCTAACTTTGAGGCTCGGAGGAGATGCTCCTCCAAATGAGTTG  
GAGATGATAGCAGTAATAATGAGACCCCGGCTCCAGCTCTGGGCCCCCATTGAGGCCG  
AGGGGGCTGCTCCGGGGCGGACTTGGTGACGTTTGGATTGGAGGATCCCTGCACTG  
CCTTCTCTGTGTTGCTCTGCTGTTTCTCTGCTGATAAACAACAACTTGGGATGAT  
CCTTTCCATTTTGATGCCAACCTCTTTTATTTTAAAGCGGCGCCTATAGT  
(SEQ. ID. NO.: 5)

FIG. 2B

SUBSTITUTE SHEET (RULE 26)

4/20

MVSYWDTGVLLCALLSCLLLTGSSSGSKLDPELSUKGTQHIMQAGQTLHLQC  
RGEAAHKWSLPEMVSKESERLSITKSACGRNGKQFCSTLTNTAQANHTGFYS  
CKYLAVPTSKKKETESAIYIFISDTGRPFVEMYSEIPEIHMTEGRELVIPCRVTSP  
NITVTLKFPDLTLPDGGKRIIWDNRKGFISNATYKEIGLLTCEATVNGHLYKTNLY  
THRQNTIIDVQISTPRPVKLLRGHTLVNCTATTPLNTRVQMTWSPDEKNKR  
ASVRRRIDQSNSHANIFYSVLTIDKMQNDKGLYTCRVRSGPSFKSVNTSVHIY  
DKAFITVKHRKQQVLETVAGKRSYRLSMKVKAFFSPVWVWLKDGLPATEKSAR  
YLTRGYSIIKDVTEEDAGNYTILLSIKQSNVFNLTATLVNVKPKIYEKAVSSFP  
DPALYPLGSRQILTCTAYGIPQPTIKWFWHPCHNHNHSEARCDFCNNNEESFILD  
ADSNMGNRIESITQRMALIEGKNKMASTLVWADSRISGIYICIASNKVGTVGRNISF  
YITDVPNGFHVNLKEMPTGEDKLSCVTNKFYRDVTWILLRTVNNRTMHYSIS  
KQKMAITKEHSITLNLTIMNVSLQDSGTYACRARNVYTGEIILQKKEITIRGEHCN  
KKAVFSRISKFKSTRNDCTTQSNVKH (SEQ. ID. NO.: 6)

FIG. 3

5/20

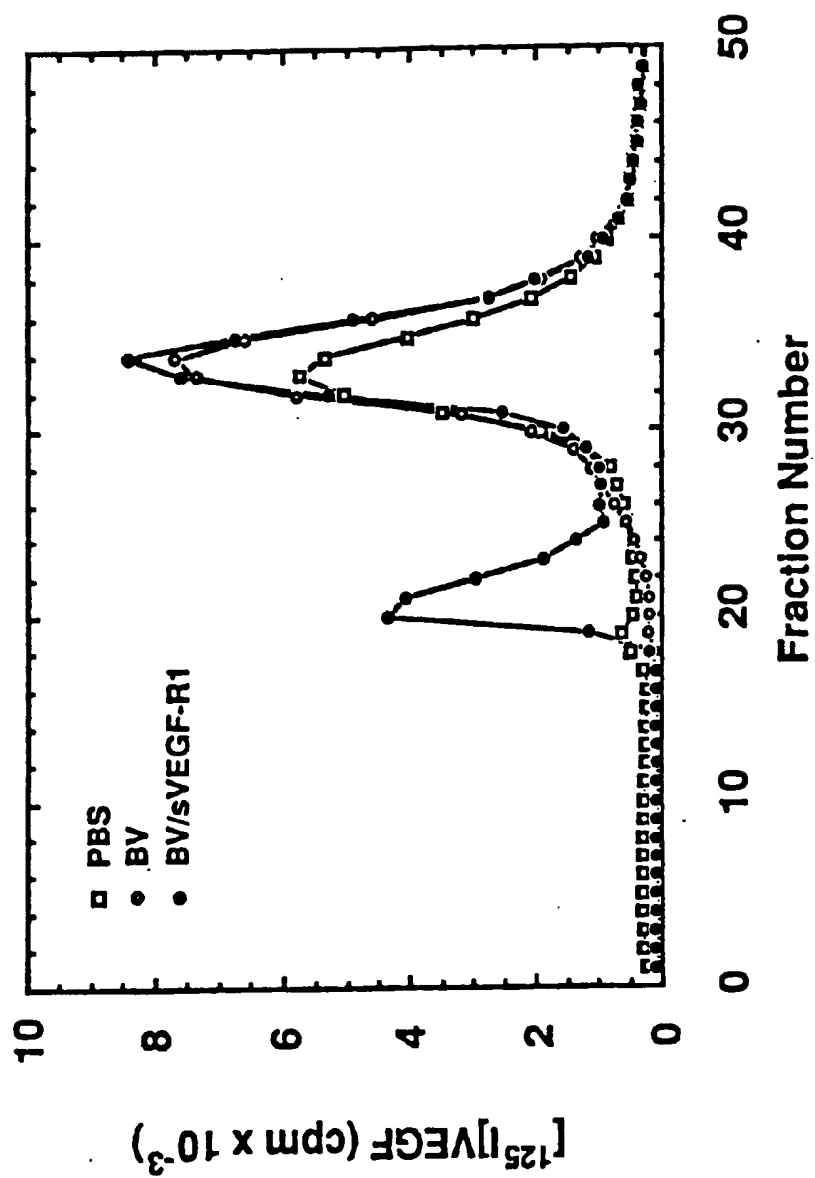
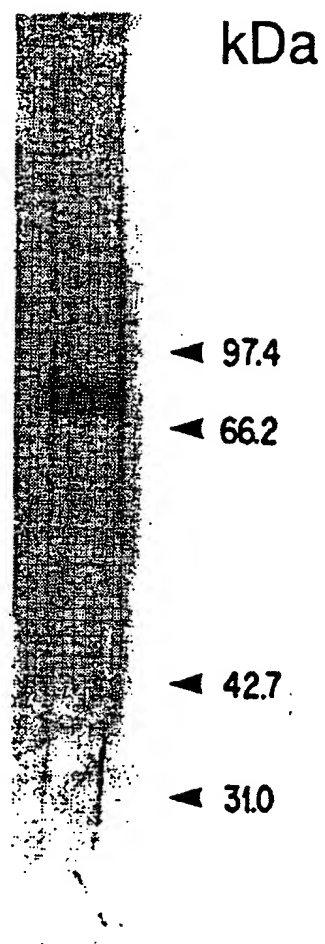


FIG. 4

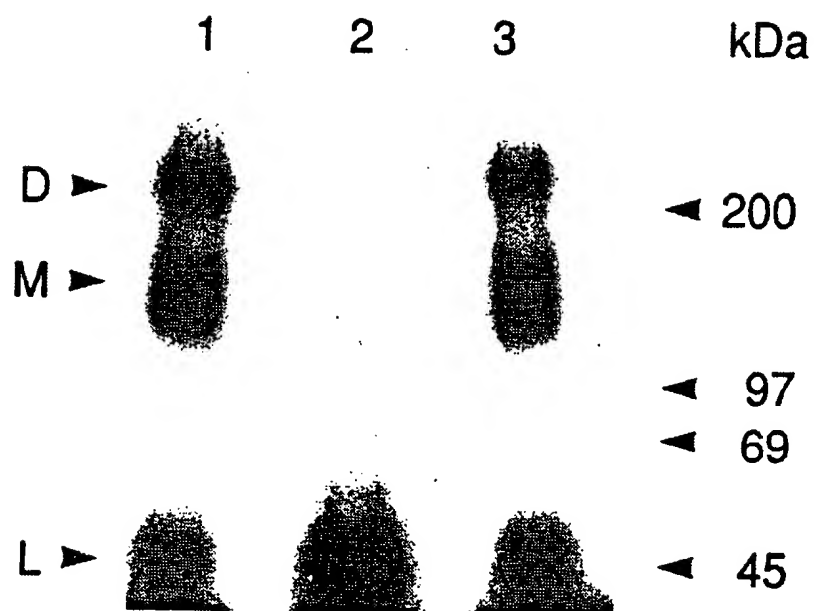
6/20



**FIG. 5**



7/20

**FIG. 6**

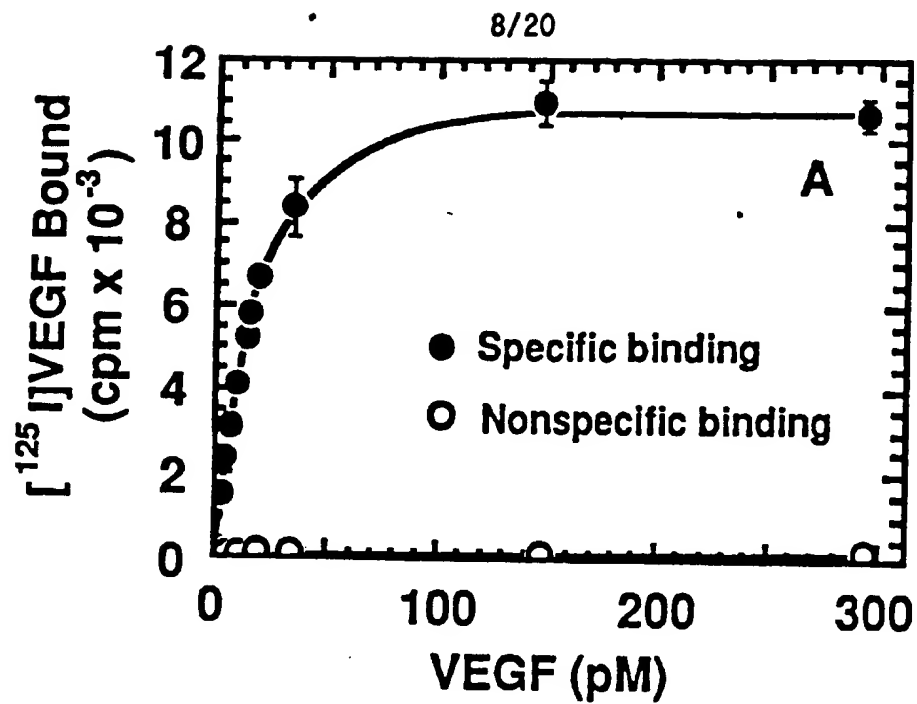


FIG. 7A

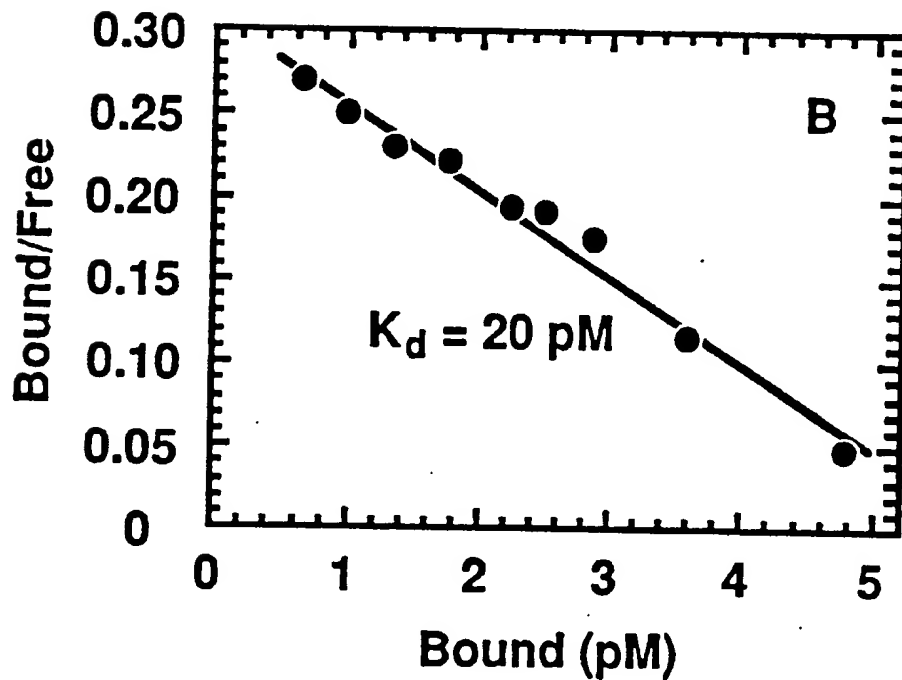


FIG. 7B

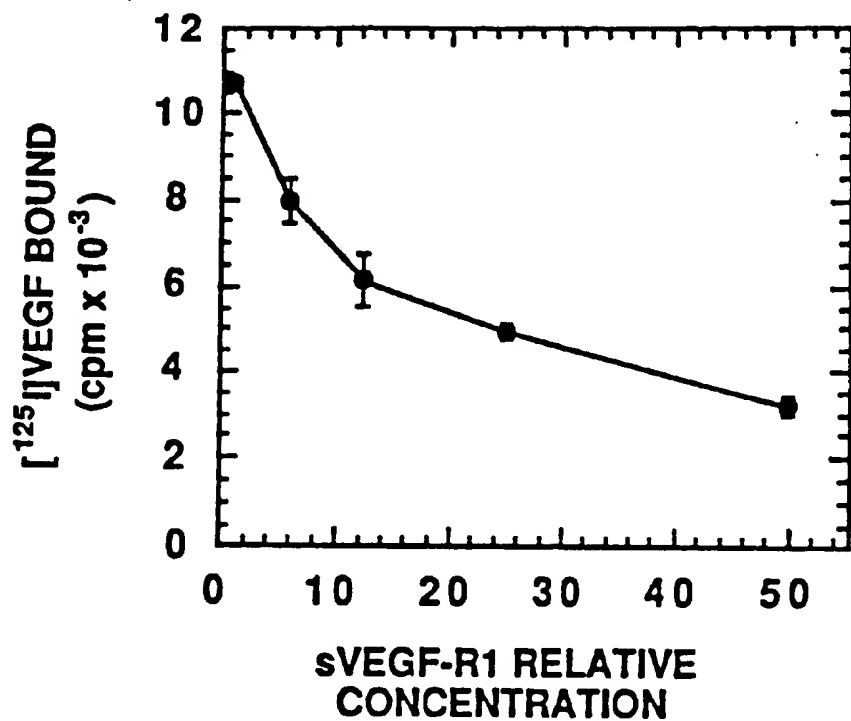


FIG. 8

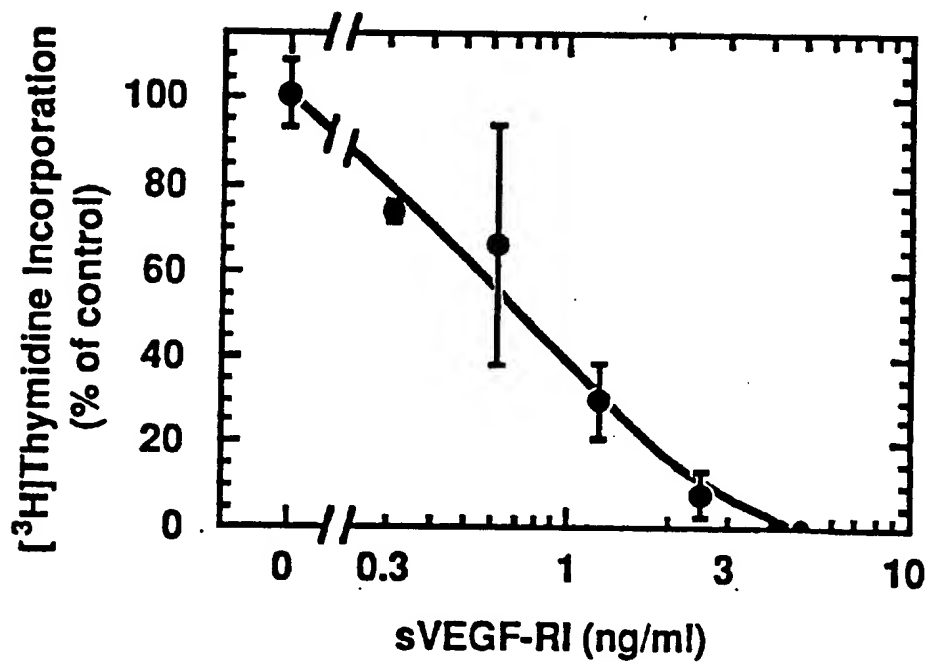


FIG. 9

SUBSTITUTE SHEET (RULE 26)

10/20

GGTGTGGTCGTCGCTTTCCTCTGCCCTGCGCCGGGCATCACTTGCGGCCCGCAGAAAGTC  
CGTCTGGCAGCCCTGGATATCCTCTCTACCGGCACCCGACGCGCCCTGCAGCCGCGGT  
CGGCGCCCGGCTCCCTAGCCCTGTGCGTCAACTGTCTGCGCTGCGGGTGCCGCGAG  
TTCACCTCCGCGCTCTCTAGACAGGCGCTGGAGAAACCGGCTCCCGAGTTC  
CGGCATTTCCGCCCGCTCGAGGTGCAGGATGCAGAGCAAGGTGCTGCGCGTCGCCCT  
GTGGCTCTGCGTGGAGACCCGGCGCCCTCTGTGGTTTGCCTAGTGTCTCTTGATCTG  
CCCAGGCTCAGCATACAAAGACATCTTACAATTAAAGCTAATACACTCTTCAAATTACT  
TGCAGGGGACAGAGGACTTGGACTGGCTTTGGCCCAATAATCAGAGTGGCAGTGAGCAA  
GGGTGGAGGTGACTGAGTGACGCGATGGCTCTTCTGTAAGACACTCACAATTCCAAAGT  
GATCGGAAATGACACTGGAGCCTACAAGTGCTTCTACCGGAAACTGACTTGGCCTCGGTC  
ATTTATGTCTATGTTCAAGATTACAGATCTCCATTTATTGCTTCTGTAGTGACCAACATGGAG  
TCGTGTACATTACTGAGAACAAACAAACTGTGGTGATCCATGTCTCGGTCATTTCAA  
ATCTCAACGTGTCACTTTGTGCAAGATACCCAGAAAGAGATTGTTCCGTGATGTAACAGAA  
TTTCTGGGACAGCAAGAAAGGCTTTACTATTCCAGCTACATGATCAGCTATGCTGGCATG  
GTCTTCTGTGAAGCAAAATTAATGATGAAGTTACCAAGTCTATTATGTACATAGTTGTCGTT  
GTAGGGTATAGGATTTATGATGTGGTTCTGAGTCCGTCATGGAATTGAACATCTGTGGA  
GAAAAGCTTGTCTTAAATTGTACAGCAAGAACTGAACATAATGTGGGATTGACTTCAACTGG  
GAATACCCCTTCTCGAAGCATCAGCATAAGAACTTGTAAACCGAGACCTAAACCCAGTCT  
GGGAGTGAGATGAGAAATTTTGTGACACCTTAACCTATAGATGGTGTAAACCGAGTGACCA

FIG. 10A

11/20

AGGATTGTACACCTGTGCAGCATCCAGTGGGCTGATGACCAAGAAGAACAGCACATTGTGCA  
GGGTCCATGAAAAACCTTTTGTGCTTTTGGAGTGGCATGGAATCTCTGGTGGAAAGCCACG  
GTGGGGAGCGTGTACAGAAATCCCTGCGAAGTACCTTGGTTACCCACCCCGAGAAATAAAT  
GGTATAAAAAATGGAATACCCCTTGAGTCCAAATCACACAAATTAAGCGGGGCATGTACTGACG  
ATTATGGAAGTGAGTGAAGAGACACAGGAAATTACACTGTATCCTTACCAATCCCATTTCA  
AAGGAGAAGCAGAGCCATGTGCTCTCTGTTGTGTATGTCCACCCAGATTGGTGAGA  
AATCTCTAATCTCTGTTGATTCTTACCATCCAGTACGGCACCACTCAACGCTGACATGTACG  
GTCTATGCCATTCTCCCGCATCACATCCACTGGTATGGCAGTTGGAGGAAAGTGCG  
CCAACGAGCCCGCAAGCTGTCTCAGTGACAAACCCATACCCCTTGTGAAGATGGAGAAG  
TGTGGAGGACTTCCAGGGAGGAAATAAATTGCCGTTAATAAAATCAATTTGCTCTAAATTGA  
AGGAAAAACAATACTGTAAGTACCTTGTATCCAAGCGGCAATGTGTCAGCTTTGTACAA  
ATGTGAAGCGGTCAACAAAGTCGGGAGAGGAGGGTGATCTCCTTCCACGTGACCAGG  
GGTCCTGAAATTACTTTGCAACCTGACATGCAGCCACTGAGCAGGAGAGCGTGTCTTTGTG  
GTGCACTGCAGACAGATCTACGTTTGAGAACCTCACATGTTGACAGCTTGGCCACAGCCTC  
TGCCAAATCCATGTGGGAGATTGCCACACCTGTTTGCAGAACTTGGATCTCTTTGGAA  
TTGAATGCCACCATGTTCTCTAATAGCACAAATGACATTTGATCATGGAGCTTAAGAAATGCA  
TCCTTGCAGGACCAAGGAGACTATGTCTGCTTGTCTCAAGACAGGAAAGACCAAGAAAGAC  
ATTGCGTGGTCAAGGCAGCTCACAGTCTAGAGCGTTAA (SEQ. ID. NO.: 16)

FIG. 10B

12/20

MQSKVLLAVALWLCVETRAASVGLPSVSLDLPRLSIQKDILTIKANTTLQITCRGQ  
RDLDWLWPNNQSGSEQRVEVTECSDGLFCKLTLPKVIGNDTGAYKCFYRETD  
LASVIYVQDYRSPFIASVSDQHGVVYITENKNKTVPICLGSISNLNVSLCARY  
PEKRFVDPDGNRISWDSKKGFTIPSYMISYAGMVCFCEAKINDESYQSIMYIVVVVG  
YRIYDVVLSPSHGIELSVGEKLVNCTARTELVNIGIDFNWEYPSSKHQHKLLVN  
RDLKTQSGSEMKKFLSTLTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRVHEK  
PFVAFGSGMESLVEATVGERVRIPAKYLGYPPEIKWYKNGIPLESNHTIKAGHV  
LTIMEVSRDGTGNYTVILTNPISKEKQSHVSVSLVYVPPQIGEKSLISPVDSYQYG  
TTQTLTCTVYAIPPPHHIHWYQLEEECANEPSQAVSVTNYPCEEWRSEDF  
QGGNKIYVKNQFALIEGKNKTVSTLVIAANVSALYKCEAVNKVGRGERVISFH  
VTRGPEITLQPDQMPTQESVSLWCTADRSTFENLTWYKLGPOPLPIHVGEPLPT  
PVCKNLDTLWKLNATMFSNSTNDILIMELKNASLQDQGDYVCLAQDRKTKKRH  
CWRQLTVLER... (SEQ. ID. NO.: 13)

7  
FIG. 11

13/20

GGTGTGGTCGCTGCGTTTCTCTGCTGCGCGGGCATCACTTGCGCGCCGAGAAAGTC  
CGTCTGGCAGCCTGGATATCCTCTCTACCGGCACCCGAGACGCCCTGCAGCCGCGGT  
CGGCGCCCGGGCTCCCTAGCCCTGCGCTCACTGTCTGCGCTGCGGGTGCCGCGAG  
TTCCACCTCCGCGCTCCTTCTCTAGACAGCGCTGGAGAAAGAACCGCTCCGAGTTC  
CGGCATTTGCGCCGGCTCGAGGTGCAGGTGACAGAGCAAGGTGCTGCGCGCTCGCCCT  
GTGCTCTGCGTGGAGACCCGCGCCCTCTGTGGGTTTGCTAGTGTCTCTTGATCTG  
CCCAGGCTCAGCATACAAAGACATACATAAAGGCTAATACAACCTCTTCAATTACT  
TGCAGGGGACAGAGGACTTGGACTGGCTTTGGCCCAATAATCAGAGTGGCAGTGAGCAAA  
GGGTGGAGGTGACTGAGTGCAGCGATGGCTCTTCTGTAGACACTCACAATCCAAAAGT  
GATCGGAAATGACACTGGAGCCTACAAGTCTTCTACCGGAAACTGACTTGGCCTCGGTC  
ATTTATGTCTATGTTCAAGATTACAGATCTCCATTTATTGCTTCTGTTAGTGACCAACATGGAG  
TCGTGTACATTACTGAGAACAAAAACAACTGTGGTGATTCATGTCTCGGGTCCATTTCAA  
ATCTCAACGTGTCACTTTGTGCAAGATACCCAGAAAGAGATTGTTCTGATGGTAACAGAA  
TTTCTGGGACAGCAAGAGGCTTTACTATTCAGCTACATGATCAGCTATGCTGGCATG  
GTCTTCTGTGAAGCAAAATTATGATGAAGTTACCAGTCTATTATGTACATAGTTGTCGT  
GTAGGGTATAGGATTTATGATGTGGTTCTGAGTCCGTCTCATGGAATTGAACATCTGTTGGA  
GAAAAGCTTGTCTTAATTGTACAGCAAGAACTGAACATAATGTTGGGATTGACTTCAACTGG  
GAATACCTTCTTCGAAGCATCAGCATAAGAACTGTAAACCGAGACCTAAAAACCCAGTCT  
GGGAGTGAGATGAAGAAATTTTGGAGCACCTTAACATATAGATGGTGAACCGGAGTGACCA  
AGGATTGTACACCTGTGCAGCATCCAGTGGGCTGATGACCAAGAAACAGACATTTGTCA  
GGGTCCATGAAAAACCTTTTGTGCTTTGGAAAGTGGCATGGAATCTCTGGTGAAGCCACG  
GTGGGGAGCGGTGCAGAAATCCCTGCGAAGTACCTTGGTTACCCACCCCGAGAAATAAAAT

FIG. 12A

14/20

GGTATAAAATGGAATACCCCTTGAGTCCAATCACACAATTAAGCGGGGCGATGTACTGACG  
ATTATGGAAGTGAGTGAAAGAGACACAGGAATTACACTGTCACTCCTTACCAATCCCATTTCA  
AAGGAGAAGCAGAGCCATGTGGTCTCTCTGTGTGTATGTCCACCCCGAGATTGGTGAGA  
AATCTCTAATCTCTCTGTGGATTCTTACCAGTACGGCACCACTCAAACGCTGACATGTACG  
GTCATGCCATTCTCTCCCGCATCACATCCACTGGTATTGGCAGTTGGAGGAAGAGTGCG  
CCAACGAGCCAGCCAAGCTGTCTCAGTGACAACCCATACCTTTGTGAAGATGGAGAAG  
TGTGGAGGACTTCCAGGGAGGAATAAATTGCCGTTAATAAAATCAATTTGCTCTAATTGA  
AGGAAAAACAACAACTGTAAGTACCCTTGTATCCAAGCGGCAATGTGTACGCTTTGTACAA  
ATGTGAAGCGGTCAACAAAGTCGGGAGAGGAGAGGGTGATCTCCTCCACGTGACCAGG  
GGTCCCTGAAATTACTTTGCAACCTGACATGCAGCCCACTGAGCAGGAGAGCGTCTTTGTG  
GTGCACCTGCAGACAGATCTACGTTTGAGAACCTCACATGGTACAAGCTTGCCCCACAGCCTC  
TGCCAAATCCATGTGGGAGAGTTGCCACACACCTGTTTGAAGAACTTGGATACTCTTTGGAA  
TTGAAATGCCACCATGTTCTCTAATAGCACAAATGACATTTTGATCATGGAGCTTAAGAATGCA  
TCCTTGCAAGCAAGGAGACTATGTCTGCCCTTGCTCAAGACAGGAAGACCAAGAAAGAC  
ATTGCGTGTGTCAGGCAGCTCACAGTCTAGAGCGTGTGGCACCACCGATCACAGGAAACCT  
GGAGAAATCAGACGACAAGTATTGGGAAAGCATCGAAGTCTCATGCAACGATCTGGGAAT  
CCCCCTCCACAGATCATGTGGTTTAAAGATAATGAGACCCTTGTAGAACTCAGGCATTGT  
ATTGAAGGATGGGAACCGGAACCTCACTATCCGCAGAGTGAGGAAGGAGGACGAAGGCCT  
CTACACCTGCCAGGCATGCAGTGTCTTGGCTGTGCAAAAGTGAGGCATTTTTCATAATAG  
AAGGTGCCAGGAAAGACGAACCTTGAAATCATATTCTAGTAGGCACGCGTGATTGCC  
ATGTTCTTCTGGCTACTTCTTGTCATCATCCTAGGGACCGTTAA (SEQ. ID. NO.: 18)

FIG. 12B



15/20

MQSKVLLAVALWLCVETRAASVGLPSVSLDLPRLSIQKDILT IKANTTLOITCRGQ  
RDLDWLWPNNQSGSEQRVEVTECSDGLFCKLTIPKVIGNDTGAYKCFYRETD  
LASVIYVYVQDYRSPFIASVSDQHGVVYITENKNKTVVIPCLGSISNLNVSLCARY  
PEKRFVPDGNRISWDSKKGFTIPSYMISYAGMVCFEAKINDESYQSIMYIVVVVG  
YRIYDVVLSPSHGIELSVGEKLVNCTARTELVGIDFNWEYPSSKHQHKKLVN  
RDLKTQSGSEMKKFLSTLTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRVHEK  
PFVAFSGMESLVEATVGERVRIPAKYLGYPPEIKWYKNGIPLESNHTIKAGHV  
LTIMEVSEKDTGNYTVILTNPISKEKQSHVVSLVYVPPQIGEKSLISPVDSYQYG  
TTQTLTCTVYAIPPP HHYHWWQLEEECANEPSQAVSVTNPYPCEEWRSEDF  
QGGNKIAVNKNQFALIEGKNKTVSTLVIQAANVSALYKCEAVNKVGRGERVISFH  
VTRGPEITLQPDMPTEQESVSLWCTADRSTFENLTWYKLGPOPLPIHVGEPT  
PVCKNLDTLWKLNATMFSNSTNDILIMELKNASLQDQGDYVCLAQDRKTKKRH  
CVVRQLTVLERVAPTITGNLENQTTSIGESIEVSCTASGNPPPQIMWFKDNETLV  
EDSGIVLKDGNRNLTIRRVKEDGLYTQACSVLGCAKVEAFFIIEGAQKTNL  
EIIILVGTTVIAMFFWLLLVIILGTV... (SEQ. ID. NO.: 15)

FIG. 13

SUBSTITUTE SHEET (RULE 26)

16/20

GGGCTACCATGGTCAGCTACTGGGACACCGGGGTCCTGCTGTGCGCGTCTCAGCTGT  
CTGCTTCTCAGAGATCTAGTTCAGGTTCAAAATTAAAGATCCTGAACCTGAGTTAAAGGC  
ACCCAGCACATCATGCAAGCAGGCCAGACACTGCATCTCCAATGCAGGGGGAAGCAGCC  
CATAAATGGTCTTTGCCTGAAATGGTGAGTAAGGAAAGCGAAAGGCTGAGCATAACTAAATC  
TGCCCTGTGGAAGAAATGGCAACAATCTGCAGTACTTTAACCTTGAACACAGCTCAAGCAA  
ACCACACTGGCTTCTACAGCTGCAATATCTAGCTACAGTAGAGCTTTGTAAGAGAGTACAGTGAA  
GAATCTGCAATCTATATATTTATTAGTGATACAGGTAGAGCTTTGTAAGAGAGTACAGTGAA  
ATCCCCGAAATTATACACATGACTGAAGGAAGGAGCTCGTCAATCCCTGCCGGTTACGTC  
ACCTAACATCACTGTTACTTTAAAAAGTTTCCACTTGACACTTTGATCCCTGATGBAAACG  
CATAATCTGGGACAGTAGAAAGGCTTCATCATATCAATGCAATGCAACGTACAAAGAAATAGGGC  
TTCTGACCTGTGAAGCAACAGTCAATGGGCAATTTGTATAAGACAAACTATCTCACACATCGAC  
AAACCAATACAATCATAGATGTCCAAATAAGCACACACGCCAGTCAAAATACCTTAGAGGC  
CATACTCTTGTCCTCAATTGTACTGCTACCACTCCCTTGAACACGAGAGTTCAAAATGACCTGG  
AGTTACCCCTGATGAAAAAATAAGAGAGCTTCCGTAAGGCGACGAA TTGACCAAAGCAATTC  
CCATGCCAACATATTTCTACAGTGTCTTACTATTGACAAATGCAAAACAAAGACAAAGGACT  
TTATACTTGTGCGTGAAGGAGTGGACCATCAATCAAACTGTAAACACCTCAGTGCATATATA  
TGATAAAGCAATTCATCACTGTGAACATCGAAACAGCAGGTGCTTGAACCCGTAGCTGGCA  
AGCGGTCTTACCGGCTCTCTATGAAAGTGAAGGCATTTCCCTCGCCGGAAGTTGTATGGTTA  
AAAGATGGGTTACCTGCGACTGAGAAATCTGCTCGCTATTGACTCGTGGCTACTCGTTAAT

FIG. 14A

17/20

TATCAAGGACGTAACTGAAGAGGATGCAGGGAATTATACAATCTTGCTGAGCATAAACAGT  
CAAATGTGTTTAAACCTCACTGCCACTCTAATTGTCAATGTGAACCCAGATTTACGAAA  
AGCGGTGTCATCGTTCCAGACCCGGCTCTCTACCCACTGGGAGAGACAAATCCTGAC  
TTGTACCGCATATGGTATCCCTCAACCTACAATCAAGTGGTTCTGGCACCCCTGTAACCATAA  
TCATTCCGAAGCAAGGTGTGACTTTTGTTCCTAATAATGAAGAGTCCCTTTATCCTGGATGCTGA  
CAGCAACATGGGAACAGAAATTGAGAGCATCACTCAGCGCATGGCAATAATAGAAGGAAAG  
AATAAGATGGCTAGCACCTTGGTTGGCTGACTCTAGAAATTTCTGGAATCTACATTTGCATA  
GCTTCCAATAAAGTTGGGACTGTGGGAAGAAACATAAGCTTTTATATCACAGATGTGCCAAAT  
GGGTTTCATGTAACTTGGAAATAATGCCGACGGAAGGAGGACCTGAAACTGTCTTGAC  
AGTTAACAAAGTTCTTATACAGAGACGTTACTTGGATTTTACTCGGACAGTTAAATACAGAAC  
AATGCACCTACAGTATTAGCAAGCAAAATAATGGCCATCACTAAGGAGCACTCCATCTTTAA  
TCTTACCATCATGAATGTTCCCTGCAAGATTCAGGCACCTATGCCTGCAGAGCCAGGAATG  
TATACACAGGGGAAGAAATCCTCCAGAAGAAAGAAATTACAATCAGAGATCAGGAAGCACCA  
TACCTCCTGCGAAACCTCAGTGATCACACAGTGGCCATCAGCAGTCCACCACCTTAGACTG  
TCATGCTAATGGTGTCCCGAGCCTCAGATCACATCTGGTTTAAACCAACCAAAATACAACA  
AGAGCCTGGAATATTATAGGACCAGGAAGCAGCAGCTGTTTATTGAAAGAGTCACAGAAG  
AGGATGAAGGTGTCATCACTGCAAGCCACCACCAAGAGGCTCTGTGGAAAGTTTCAGC  
ATACCTCACTGTTCAAGGAACCTCGGACAAGTCTAATCTGGAGCTGATCACTCTAACATGCA  
CCTGTGGCTGCGACTCTCTCTGGCTCCTATTAAACCCTCCTTATCTAA (SEQ. ID. NO.: 17)

FIG. 14B

SUBSTITUTE SHEET (RULE 26)

18/20

MVSYWDTGVLLCALLSCLLLTGSSSGSKLDPELSLKGTHIMQAGQTLHLQC  
RGEAAHKWSLPPEMVSKESERLSITKSACGRNGKQFCSTLTLNTAQANHTGFYS  
CKYLAVPTSKKKESESAYIFISDTGRPFVEMYSEIPEIIHMTGRELVIPCRVTSP  
NITVTLKKFPLDTLIPDGKRIIWDSRKGFIISNATYKEIGLLTCEATVNGHLYKTNLY  
THRQNTNIIDVQISTPRPVKLLRGHTLVLNCTATTPLNTRVQMTWSYPDEKNKR  
ASVRRRIDQSNHANIFYSVLTIDKMQNKDKGLYTCRVRSGPSFKSVNTSVHIY  
DKAFITVKHRKQQVLETVAGKRSYRLSMKVKAFPSPEVWVWKDGLPATEKSAR  
YLTRGYSLLIKDVTEEDAGNYTILLSIKQSNVFNLTATLIVNVKPOIYEKAVSSFP  
DPALYPLGSRQILTCTAYGIPQPTIKWFWHPCNHNHSEARCDFCNNNEESFILD  
ADSNMGNRIESITORMAIIIEGKNKMASTLVVADSRISGIYICIASNKVGTVGRNIF  
YITDVPNGFHVNLKMPTEGEDLKLCTVKNFLYRDVTWILLRTVNNRTMHYSIS  
KQKMAITKEHSITLNLTIMNVSLQDSGTACRARNVYTGEELQKKEITIRDQEAP  
YLLRNLSDHTVAISSSTLDCHANGVPEPQITWFKNNHKIQQEPGIIILGPGSSSTLF  
IERVTEEDGVYHCKATNQKGSVESSAYLTVQGTSDKSNLELTLTCTCVAATLF  
WLLLTLLI (SEQ. ID. NO.:14)

FIG. 15

SUBSTITUTE SHEET (RULE 26)

19/20

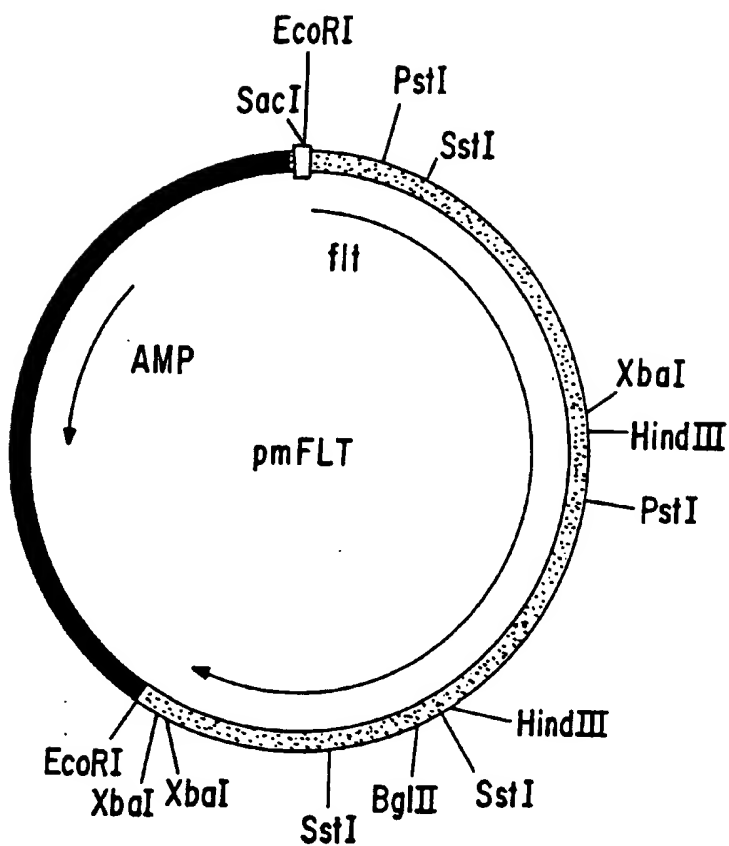


FIG. 16

20/20

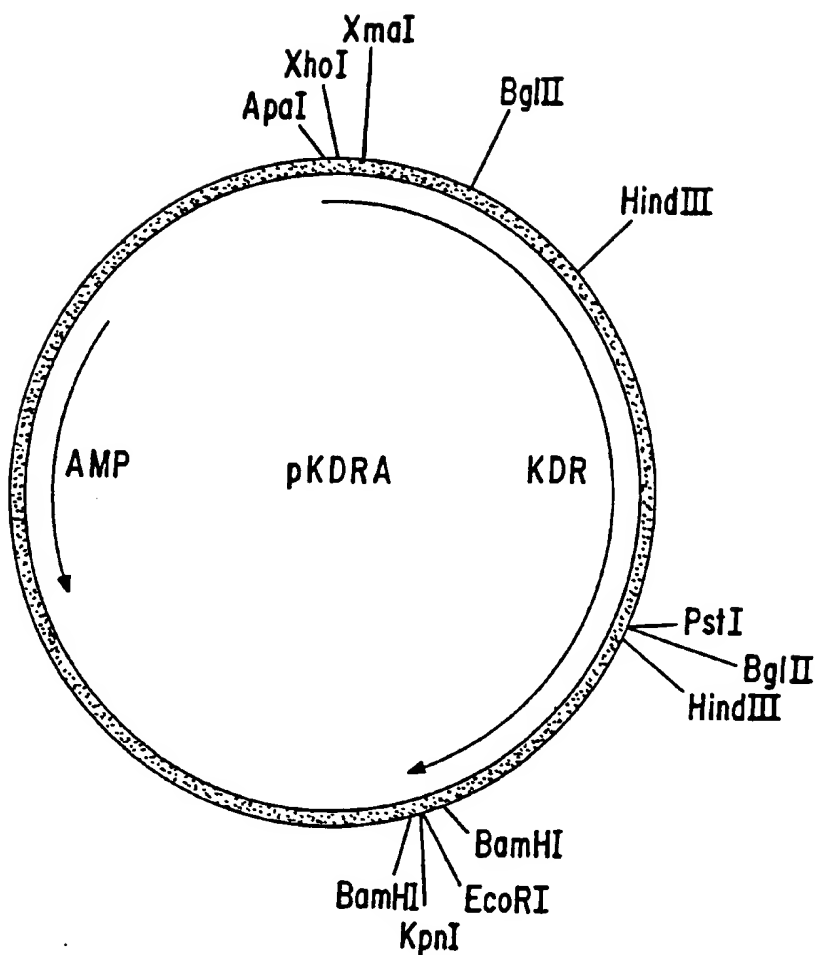


FIG. 17

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US94/01957

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(S) : C07K 13/00; C12P 21/00; C12N 5/00, 15/00

US CL : 435/69.1, 240.1, 320.1; 530/350; 536/23.1

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/69.1, 240.1, 320.1; 530/350; 536/23.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Medline, Biosis, WPI

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Journal of Cellular Physiology, Volume 149, Number 1, issued October 1991, Bikfalvi et al, "Interaction of Vasculotropin/Vascular Endothelial Cell Growth Factor with Human Umbilical Vein Endothelial Cells: Binding, Internalization, Degradation, and Biological Effects", pages 50-59, see abstract.	1
Y		14, 15, 18
Y	Science, Volume 255, issued 21 February 1992, De Vries et al, "The fms-Like Tyrosine Kinase, a Receptor for Vascular endothelial Growth Factor", pages 989-991, see abstract and fig. 1.	1-18

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be part of particular relevance	X	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*E* earlier document published on or after the international filing date	Y	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	A*	document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means		
*P* document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

12 MAY 1994

Date of mailing of the international search report

JUN 03 1994

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

Sally P. Teng

Telephone No. (703) 308-0196

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Oncogene, Volume 5, issued 1990, Shibuya et al, "Nucleotide Sequence and Expression of a Novel Human Receptor-Type Tyrosine Kinase Gene (flt) Closely Related to the fms Family", pages 519-524, see abstract and page 521.	1-18
Y	Biochemical and Biophysical Research Communications, Volume 187, Number 3, issued 30 September 1992, Terman et al, "Identification of the KDR Tyrosine Kinase as a Receptor for Vascular Endothelial Cell Growth Factor", pages 1579-1586, see summary and page 1583.	1-18